Journal of
DAIRY SCIENCE

Vol. 43 April, 1960 Number 4

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PLAN TO ATTEND THE 55TH ANNUAL MEETING
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AMERICAN DAIRY SCIENCE ASSOCIATION
in the Scenic Cache Valley at
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JUNE 19–22, 1960

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Advertising plates, copy, insertion orders, contracts, and requests for information relating to advertising in the Journal should be addressed to the Journal of Dairy Science in care of The Garrard Press, 510-522 North Hickory Street, Champaign, Illinois.

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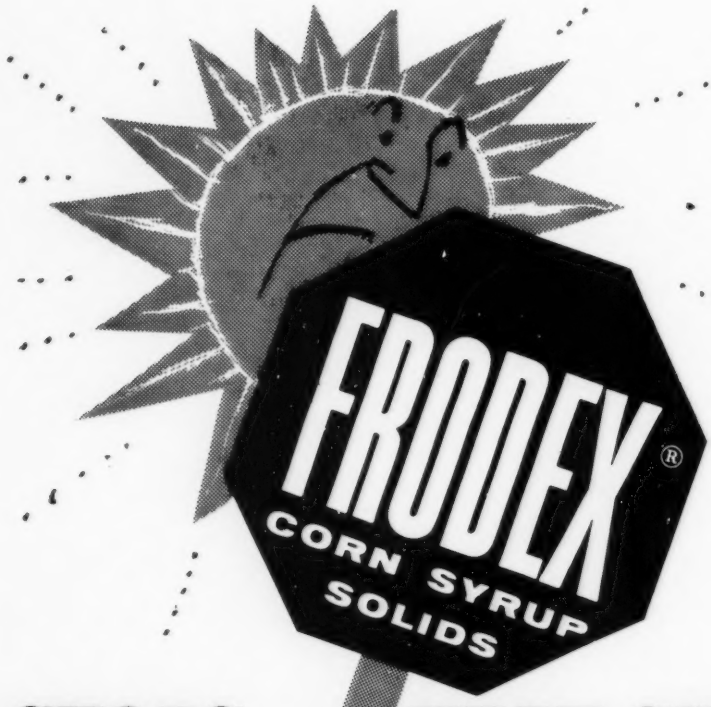

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PEOPLE AND EVENTS

Otto Frederick Hunziker Memorial

The University community, the Dairy Industry, the nation and the world, mourn the passing of O. F. HUNZIKER on Sunday, November 16, 1959, at the age of eighty-five. He served the Dairy Industry well as a scientist and factory consultant.

O. F. Hunziker

He was born in Zurich, Switzerland, on December 25, 1873, the son of Karl Otto and Louise Hunziker. The father was originally a minister and later, professor of education and pedagogy at the University of Zurich. The childhood days of the two brothers and two sisters were spent in Goldbach, a small town near Zurich. Otto graduated from the Agricultural College nearby when he was 19 yr. old. His longing to go to the United States was fulfilled when he settled on a dairy farm near Attleboro, Massachusetts, in 1893.

Realizing his handicap with the English language and longing for business training, he attended a business college at Providence, Rhode Island, and graduated in 1896. He returned to Switzerland in 1898 and found his homeland to be a great dairy country calling for the solution of many technical problems. After further study, he again crossed the Atlantic at the age of 25 and enrolled at Cornell University. After graduating in dairy with honors in 1900, he sought commercial experience in a creamery in New York for 3 mo. and returned to Cornell University to earn a Master's Degree in 1901 and to serve as assistant in charge of Dairy Bacteriology for 1 yr.

He received additional practical training in a condensed milk plant at Ellicottville, New York, where he worked with dairy farmers and in the plant. Here he equipped and operated a research laboratory. Solving dairy manufacturing problems through research became his domain.

He became an American citizen while employed at Ellicottville. Here he married Florence Belle Burne in 1905, a marriage destined to extend over 55 yr. Surviving are the widow, Mrs. F. Belle Hunziker, LaGrange, Illinois; two sons: Walter B., Tinley Park, Illinois, and Otto F. Jr., Lakewood, Crystal Lake, Illinois, and three daughters: Mrs. R. A. Tipple, Dallas, Texas; Mrs. C. D. Galvin, Orinda, California; and Mrs. M. E. Kerr, Oakland, California.

In 1905 he accepted a position in the Dairy Department at Purdue University and was made head of the department in 1907. The research program under his direction established the accuracy of the glassware and provided standard methods for the Babcock fat test of milk and cream. He searched for information of practical benefit to the Dairy Industry and won recognition in the fields of butter making, condensed milk, and dried milk. As a scientist of dairy manufacturing he made commercial butter making a process with a scientific foundation for every step.

Dr. Hunziker is known throughout the world as the author of several well-known books which have served as textbooks in the agricultural colleges in the United States and in other dairy countries. The Butter Industry, first published in 1920, was revised in 1927 and 1940. His Condensed Milk and Milk Powder, first published in 1914, was revised in 1918, 1920, and 1926. During his academic career at Purdue, he published over 50 bulletins, leaflets, and scientific treatises dealing with the solution of problems on dairy farms and in dairy plants. His books, bulletins, leaflets, and scientific articles are recognized as a distinct contribution to the knowledge in his fields.

Dr. Hunziker planned Smith Hall, which was built in 1913 to provide additional space and facilities for administration, teaching, research, and extension work in dairy. The new dairy building also included an annex for a Purdue University Creamery.

The importance of the practical application of the scientific facts led him to take charge of the butter department of the Blue Valley Creamery Company, with headquarters in Chicago, in 1917. Here he established a research program and a laboratory service for 22 creameries and two milk plants. He introduced new and more efficient methods of processing. His industrial accomplishments equalled his academic attainments. After 1939 he served the dairy industry as a dairy consultant, lecturer, author, and publisher.

He was a charter member of the American Dairy Science Association and served as the third president of the organization in 1910. The association gave him an honorary scroll in 1934 and an honorary membership in 1942.

He received an honorary doctoral degree in science at Purdue University in 1932 in appreciation of his conscientious and skillful work as professor and chief of the Dairy Department from 1905-1916, and as a testimonial of his achievement as a scientist, teacher, and author.

He was chosen as one of the ten Master Minds in Dairy in 1930 when two farm journal editors were prompted to make an exhaustive survey to determine what men were responsible for the important status of the Dairy Industry. While 123 men were mentioned for the honors, only ten master minds were chosen, Babcock, Hoard, Eckles, DeLaval, McCollum,

Pasteur, Haecker, Borden, Henry, and Hunziker. Dr. Hunziker stands out as a physically and intellectually rugged servant among the pioneers of the dairy industry. His accomplishments, affiliations and honors reflect his great mission to serve many. The present scientists, leaders, and operators on college campuses and in dairy plants, and others who knew and loved Dr. Hunziker, feel a distinct loss in the passing of such a successful scientist who dedicated over 60 yr. of his life to making a better dairy industry.

WELCOME TO UTAH FROM GOVERNOR G. D. CLYDE AND RESPONSE BY PRESIDENT A. C. FAY

State of Utah
Office of the Governor
Salt Lake City

December 31, 1959

Mr. A. C. Fay, President
The American Dairy Science Association
12458 North Bay Shore Drive
Keystone Island
North Miami, Florida.

Dear President Fay:

I was very happy to learn that the American Dairy Science Association will hold its 55th annual meeting in Utah at our Utah State University. I am very proud of our Land Grant College at Logan and I know that President Daryl Chase and the University staff will take good care of you. I am delighted that President Chase is making it possible for you to enjoy a concert by our famous Mormon Tabernacle Choir.

As a dairy state, we produce less than one per cent of the Nation's milk supply, but we rank high in the quality of our dairy products and the leadership that makes it possible.

On the way to Logan or as you leave your Conference, I invite you to see our state. Visit in Salt Lake City at the State Capitol Building and see our unusual relief maps and exhibits of agriculture, industry, mining, history, and arts. Then tour our capital city and see such places as the LDS Temple Square, with its unique museum and beautiful grounds. You will also want to see the largest open-faced copper mine in the world and the Great Salt Lake.

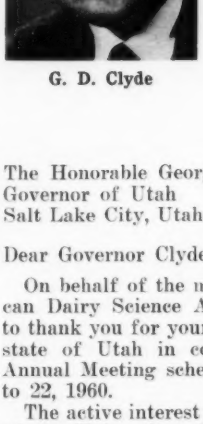
A trip through the state will reveal our magnificent scenery.

I hope to have the opportunity to greet you and your great organization at your Logan Conference.

Welcome to Utah.

Yours sincerely,

(Signed) GEORGE D. CLYDE
Governor



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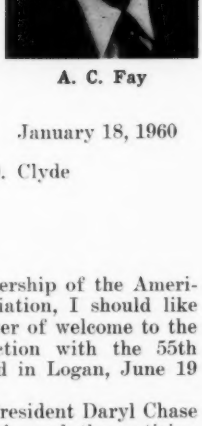


A. C. Fay

January 18, 1960

The Honorable George D. Clyde
Governor of Utah
Salt Lake City, Utah

Dear Governor Clyde:

On behalf of the membership of the American Dairy Science Association, I should like to thank you for your letter of welcome to the state of Utah in connection with the 55th Annual Meeting scheduled in Logan, June 19 to 22, 1960.

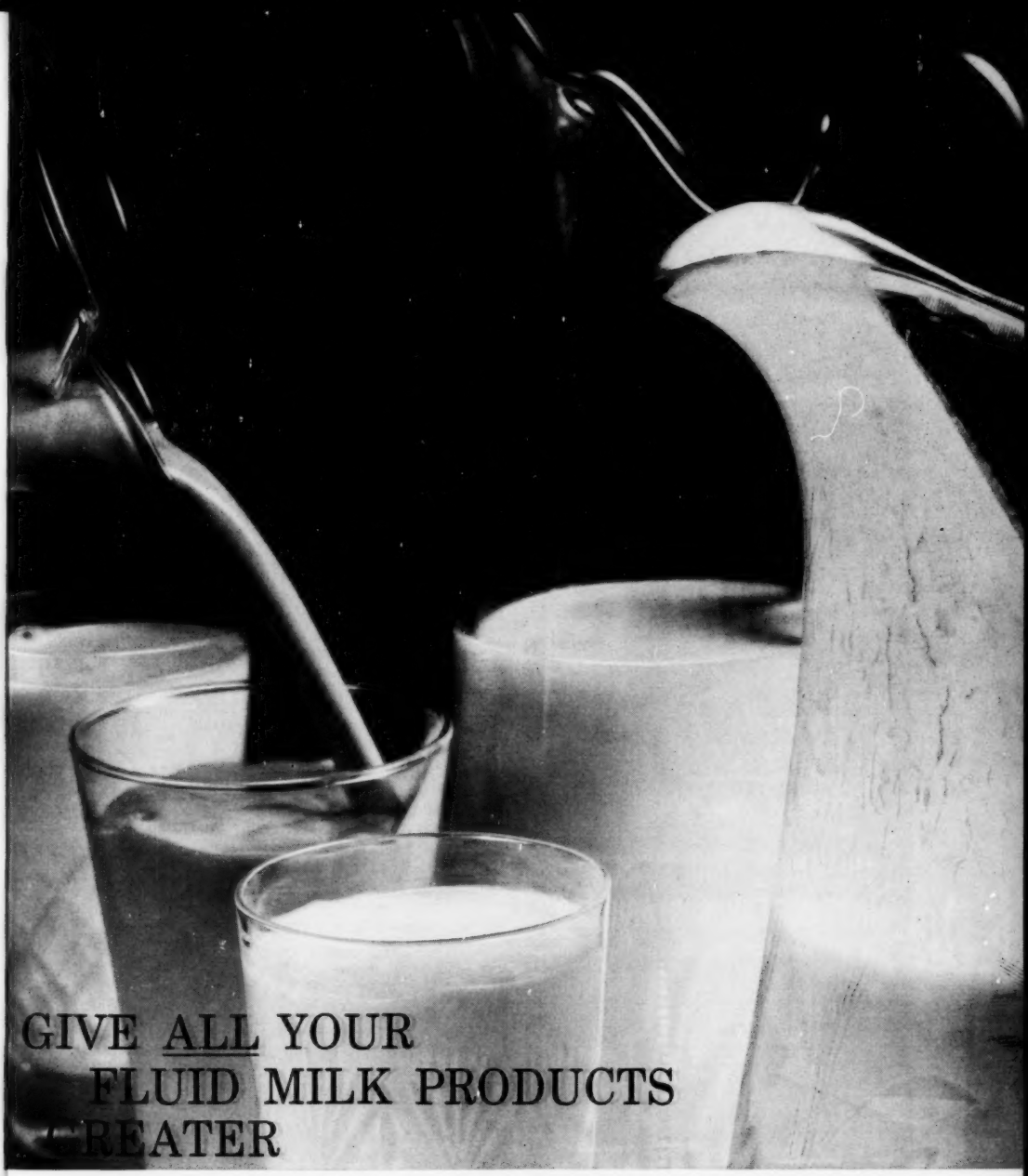
The active interest of President Daryl Chase of the University of Utah, and the untiring efforts of Professor Arthur J. Morris and his associates of the Dairy Industry Department, in making plans for housing and entertainment reflect the wholesome hospitality for which the people of your state are famous.

Preliminary indications give promise of a record attendance. The lure of the unsurpassed scenery of Utah, and the opportunity to hear the world-famous Mormon Tabernacle Choir, coupled with a strong scientific program, provide the incentive for combining this trip with a vacation for the members and their families.

The warmth of your welcome to the state of Utah is deeply appreciated.

Yours truly,

A. C. FAY,
President, American Dairy Science Association



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Ohio State News

R. M. KOTTMAN, Dean of the College of Agriculture, Forestry, and Home Economics at West Virginia University, has been appointed dean of the Ohio State University College of Agriculture and Home Economics, and director of the Ohio Experiment Station at Wooster, effective June 1. He will succeed DEAN L. L. RUMMELL who has held the dual position since 1948 and until his retirement December 31, 1959.

Dr. Kottman received his B.S. degree in Animal Husbandry from Iowa State University, 1940; his M.S. degree in Genetics from the University of Wisconsin, 1948; and his Ph.D. degree in Animal Breeding from Iowa State University, 1952. From 1940-41 Dr. Kottman was employed by the Ralston-Purina Co., St. Louis, Mo. He served in the U. S. Army during World War II, from 1941-46, being separated from active duty as a major.

Prior to being appointed to the deanship at West Virginia University, Dr. Kottman served at Iowa State University as Assistant Professor of animal husbandry, 1946-1950; assistant professor and assistant to the dean of agriculture, 1950-51; associate professor and assistant dean of agriculture, 1951-1954; and professor and associate dean in charge of the agricultural teaching program, 1954-58.

During the past year, Dr. Kottman served as secretary of the Division of Agriculture of the American Association of Land-Grant Colleges and State Universities and as Chairman of the division's Resident Instruction Committee on Organization and Policy. He is currently chairman of the Agribusiness Subcommittee, and was recently elected to the Executive Committee of the association's Division of Agriculture.

Approximately 450 people attended the 27th Annual Dairy Technology Conference at Ohio State University, February 9-11. The general areas covered and themes of discussion in each area were as follows:

Field work: Basic Industry Problems in Ohio, New Practices and Methods, Basic Problems of Bulk Tank Change and Operation, Integrating Methods and Economics.

Dairy plant management and operations: Some Basic Factors Affecting Management Decisions, Aids and Hindrances in Milk Distribution.

Engineering and processing: Advances in Heat Processing, Paper Packaging of Fluid Products.

Quality control and cultured products: Advances in Quality Control, Means to Better Cultured Products.

Ice cream operations: Let's Look at Ourselves, The Vanilla Ice Cream Situation.

Visiting speakers included:

R. J. ABELL, Weyerhaeuser Timber Co., Camden, N. J.
S. ALFEND, Federal Food & Drug Administration, Cincinnati
H. ANDERSON, Babcock Dairy Co., Toledo
B. C. BOTTS, Sealtest Foods, Inc., Cleveland
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H. G. GEYER, State Veterinarian, Columbus
J. GOOD, Pickerington Creamery, Pickerington
T. K. HAMILTON, The Diamond Milk Company, Columbus
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J. C. WHITE, Cornell University, Ithaca, New York

R. E. Feeney

R. E. Feeney Joins University of California

R. E. FEENEY, nationally known for his research in protein chemistry, joined the University of California, in the Department of Food Science and Technology, at the Davis campus March 15. He has headed the Department of Biochemistry and Nutrition at the

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University of Nebraska since 1953. He will teach courses in the biochemistry of food proteins and do basic research work emphasizing the proteins of milk, eggs, and meat.

Dr. Feeney will become part of a closely working team of biochemists in several departments on the Davis campus working on basic research in protein chemistry. Research projects will be planned on the application of protein chemistry to the processing, manufacture, storage, and distribution of foods, including the effects of freezing, concentrating, and drying, and heat processing.

Dr. Feeney earned the Bachelor of Science degree in chemistry at Northwestern University in 1938; the Master of Science and Doctor of Philosophy degrees in biochemistry at the University of Wisconsin, in 1940 and 1942. He then held a postdoctoral position as a research associate at the Harvard Medical School.

After World War II, in which he served as a food and nutrition officer, he returned to California in 1946 as a biochemist in the USDA Western Regional Research Laboratory at Albany. From there he went to the University of Nebraska, in 1953.

He is the author or co-author of about 40 scientific papers, and the co-holder of three patents. He has been active in the American Chemical Society and the National Science Foundation, and he has received several research grants from the National Institutes of Health.

Dr. Feeney's early career was in the field of milk products. He started as a part-time route man for the Borden Company in Chicago in 1924 and later was foreman of a milk processing plant.

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North Carolina News

J. P. EVERETT joined the Dairy Husbandry Staff at North Carolina State College February 1, 1960. He replaces C. D. GRINNELLS, who retired in 1958. Mr. Everett is a native of Georgia, took his undergraduate work at Auburn University, his Master's at Kentucky, and is currently finishing his Doctorate at Michigan State University. During his graduate training, he worked in the field of roughage utilization and will continue in this phase of the research program at North Carolina State College, as well as do some teaching.

E. J. STONE has returned to Louisiana State University after spending his sabbatical leave doing graduate work toward his Doctorate at North Carolina State College.

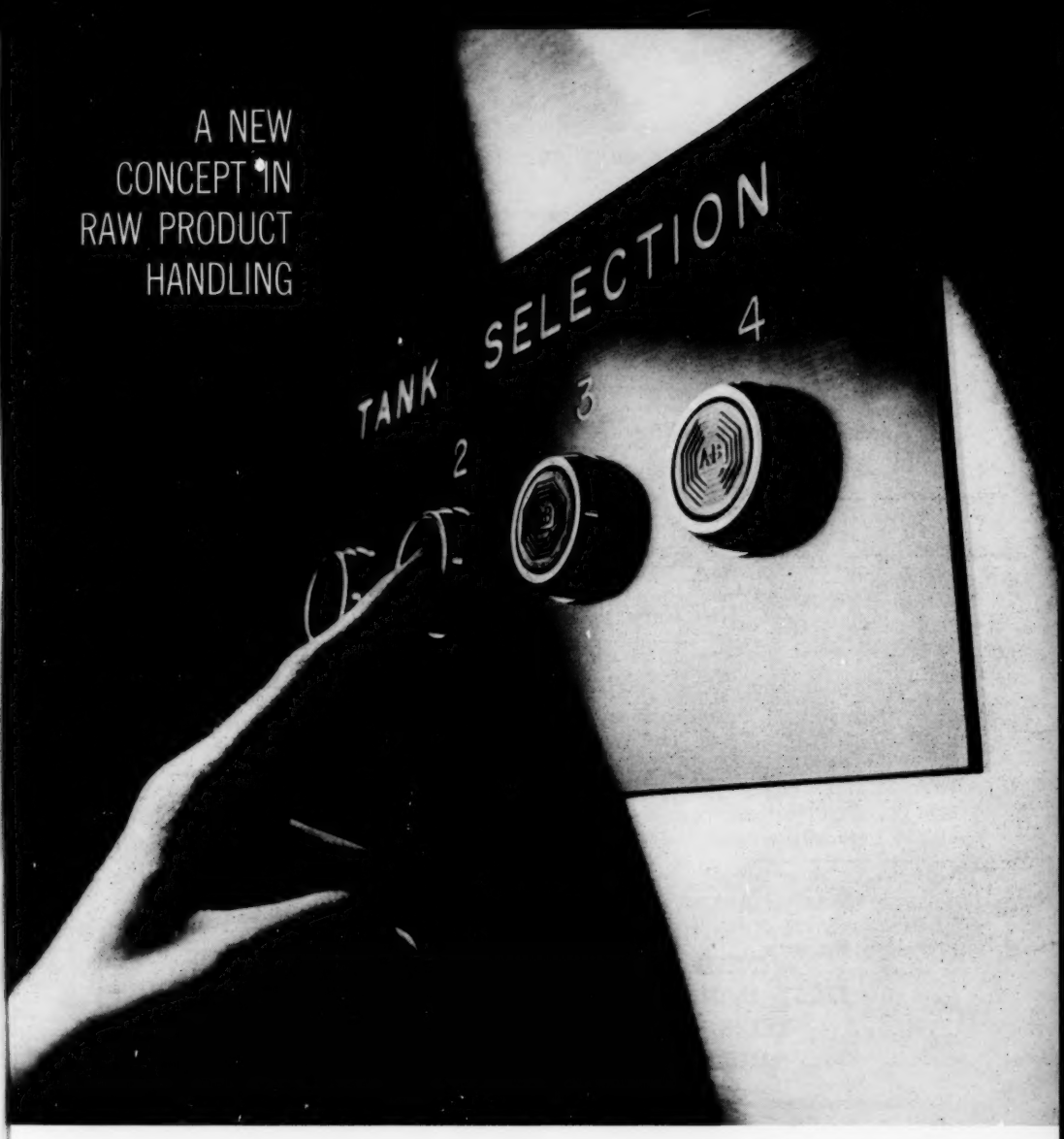
FRED HATHORN, Southwestern Louisiana Institute, Lafayette, Louisiana, has returned to North Carolina State to continue his work toward the Doctorate in Dairy Husbandry.

Plans are being drawn for an $830,000 addition to Polk Hall which houses the Animal Industry Department. This addition will more than double the space and facilities for the expanding research program and the increased student load. Construction should begin sometime during 1960. The money was made available by a bond issue voted upon by the citizens of North Carolina in November, 1959.

West Virginia University

The 19th Annual West Virginia University Dairy Short Course was held February 22-24 at Jackson's Mill, Weston. One full day's program on forage production from the seed to the plant was interestingly given. Station agronomists and agricultural engineers were speakers.

Another full day was spent on discussions on feeding the calf and the cow. Out-of-state speakers for the event included J. C. NAGEOTTE, Emeritus Professor of Dairy Extension, Pennsylvania State University and now a dairy consultant, who gave an entertaining talk, with slides, on his trip to Western Europe. He discussed in a practical way the prevention of mastitis. J. T. REID, Cornell University, talked in a thought-provoking manner about feeding forage to dairy cows. GEORGE HOPSON of the DeLaval Company discussed milking and the care of milking machines, in his interesting, practical way.



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The key to push-button control of raw product handling is Cherry-Burrell's exclusive Load-A-Matic, which consists of three basic components — load cells, automatic valves and a control panel.

With these three pieces of equipment you can unload tank trucks, load and unload storage tanks, take instant inventory, load-out and standardize — automatically with the push of a button. And one man can control all of these operations.

ECONOMICAL STEP-BY-STEP INSTALLATION

Bringing raw product handling under the push-button control of one man is not a costly procedure. Cherry-Burrell engineers can design the system for economical step-by-step installation. This method keeps new equipment investment to a minimum. And, at the same time, your in-plant profit increases as the equipment you do install reduces man-hours, improves efficiency in a given operation.

PLAN WITH CHERRY-BURRELL

Installing a Cherry-Burrell automated raw product handling system calls for careful planning. The program must be laid out precisely — the system accurately designed to your requirements. Each piece of equipment must be looked at in the light of its place in the over-all system.

A Cherry-Burrell Sales Engineer can help you start this vital planning now . . . planning that results in an automated system best suited to your present needs and one which can be easily and economically expanded in the future. Get in touch with a Cherry-Burrell Sales Engineer today.

Write for a free copy of Cherry-Burrell's new Automated Systems Bulletin

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Profitable Brands Start with Cherry-Burrell

The closing talk by I. D. PORTERFIELD, head of the West Virginia University's Dairy Department, discussed the future of dairying in West Virginia and cited studies now in progress.

Denmark Confers Its Highest Honor on G. H. Wilster

G. H. WILSTER, Professor Emeritus of Dairy Technology at Oregon State College, has received highest honors from his native land, Denmark.

By order of His Majesty, King Frederik IX of Denmark, Wilster has been presented the Knight Cross of the Order of Dannebrog. The recognition was given Wilster for his world-wide contributions in the dairy industry. Wilster was born in Denmark and lived there until he was 18.

G. H. Wilster

Wilster went to Denmark in 1955-56 as a Fulbright lecturer at the Danish Royal Agricultural College, Copenhagen. During his stay in Denmark, he lectured in several neighboring European countries under sponsorship of the U. S. State Department. He also translated a Danish dairy book into English for use in teaching throughout the world.

Wilster is author or joint author of some 200 publications, including three widely used textbooks, and is internationally known for his research on butter, cheese, and concentrated milk products.

After his retirement from the Oregon State College staff after 29½ yr., Wilster accepted an assignment to India for the Food and Agriculture Organization of the United Nations. He was technical advisor for Dairy Society International at the Calcutta Trade Fair last summer.

Wilster returned to this country earlier this year and attended the recent 49th annual convention of Oregon Dairy Industries at the Oregon State College. He received the Danish honor en route to this country from India. Wilster has been asked to return to India by FAO and plans to do so later this year.

The Wilsters make their home at Lakeside, San Diego County, California.

—from the *Gazette-Times*, Corvallis, Oregon, February, 1960

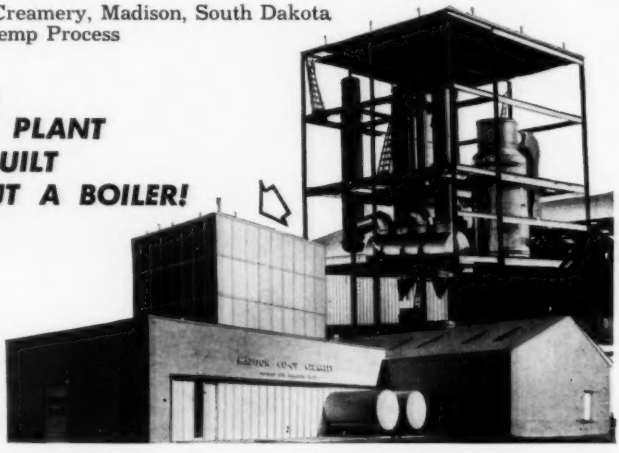
Sherman Appointed Industrial Relations Manager at Cherry-Burrell Corp.

Appointment of N. R. SHERMAN as Manager of Industrial Relations at Cherry-Burrell Cor-

When Farmer's Cooperative Creamery, Madison, South Dakota installed the Mojonnier Lo-Temp Process in its entirely new plant, it became the

WORLD'S FIRST DAIRY PLANT TO BE BUILT WITHOUT A BOILER!

No boiler — no steam — no water — no water disposal problem is the modern day achievement of the Mojonnier Lo-Temp Evaporator — Spray Dryer System. Using the heat pump principle, the system produces 825 pounds of premium grade nonfat dry milk per hour at Farmer's Cooperative on a continuous production basis. Features include in-place cleaning, retention of maximum Whey Protein Nitrogen values, pushbutton operation, and lowest production cost per pound of dried product. Milk is evaporated at temperatures below the cow's body temperature.



Above: A primary feature is that the steel framework which supports the Lo-Temp Evaporator and Spray Dryer equipment also supports the low cost outer sheathing. Arrow points to completed structure.

Full details are in Bulletin 372-18. Write for it today
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Mojonnier LO-TEMP PROCESS
QUALITY ENGINEERED FOR ECONOMY

poration was announced recently by E. M. GILES, Director of Personnel and Public Relations, and Patent Counsel for the company.

N. R. Sherman

In the newly created post, Sherman will assist in developing corporate labor relations policies at Cherry-Burrell. He will have jurisdiction over the negotiations of collective bargaining agreements in company plants. He will manage corporate recruitment and aptitude testing, hiring in Cedar Rapids, as well as administer the employee training program and safety program.

Sherman received his B.A. degree at Marquette University in Milwaukee. He was formerly with a Midwest food processor in industrial relations work.

Policy Committee Meets at Columbus, Ohio

The Policy Committee of the American Dairy Science Association enjoyed the hospitality of J. F. CAVANAUGH in the American Jersey Cattle Club offices for their meeting

POLICY COMMITTEE IN ACTION

Left to right: I. W. Rupel, College Station, Texas; J. H. Erb, Columbus, Ohio; H. F. Judkins, White Plains, New York; I. A. Gould, Columbus, Ohio; L. A. Moore, Beltsville, Maryland; and Floyd Arnold, Ames, Iowa.

on February 23 and 24. The Policy Committee is composed of W. V. PRICE, Chairman, University of Wisconsin; I. A. GOULD, Ohio State; I. W. RUPEL, Texas A & M; L. A. MOORE, Animal Husbandry Research Division; FLOYD ARNOLD, Iowa State University; J. HOFFMAN ERB, The Borden Company; H. F. JUDKINS, Secretary-Treasurer, A.D.S.A.

All members were present except W. V. Price, who was taken suddenly ill the night before the meeting. I. A. Gould did an excellent job as temporary chairman.

Items discussed by the Committee were:

1. Future planning for the business organization of the Association.
2. Clarification of responsibilities of the Journal Management Committee.
3. Public relations for the Association.
4. International relationships in dairy sciences.

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EASY TO USE. Iodine sanitizers are formulated especially for treatment of dairy utensils and equipment. Leading manufacturers offer iodine sanitizers and detergent-sanitizers as liquids, powders or tablets.

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5. Work of the Resolutions Committee.
6. More teaching awards.
7. Academic freedom.
8. Recognition for past-presidents.
9. Modernizing the constitution.

The recommendations of the Policy Committee will be discussed by the Board of Directors at the Annual Meeting at Utah State University in June, 1960.

Completed Thesis

Ph.D. Degree

ENRIQUE LUIS PINEIRO—The use of new antioxidants in frozen dairy products. Louisiana State University, Baton Rouge.

Dairy Technology Societies

Atlanta—A meeting of special interest to all managers, production personnel, and sales managers was held March 7. L. J. HAYHURST, Research and Development Division, National Dairy Products Research Laboratory, Glenview, Illinois, spoke on Latest Ideas in the Packaging of Dairy Products and the Role of Research in Promoting Dairy Foods Through Innovations.

Central Illinois—The subject Are You Missing Sales with Your Chocolate Ice Cream? was discussed by J. R. WRIGHT, Ambrosia Chocolate Company of Milwaukee, Wisconsin, at the group's March 9 meeting at the Orlando Hotel, Decatur. This gave some of the reasons why only 16-20% of the total gallonage of ice cream manufactured is chocolate. Movies were also shown—on the growing of chocolate beans in Africa.

Central Michigan—March 16 meeting—High Life Inn—Saginaw—featured J. HOFFMAN ERB of the Borden Company as speaker. Subject of his address was Keys to Plant Operation. His talk was not confined to ice cream plant operation but to plant operation in general.

Detroit—TONY MERLLO of the Merllo Steam Equipment Company of Detroit spoke at the March 14 regular meeting of this society. Topic discussed was: Purgers—using a cut-a-way model and slides for illustration.

Greater St. Louis—H. E. WITMIRE of the Witmire Research Laboratories, Inc., St. Louis, Missouri, spoke at the February 16 meeting on Comments on the Elimination of Toxic Compounds Through the Milk of Dairy Cows. Mr. Witmire's comments related to the recent activity of the Federal Food and Drug Administration in the field of antibiotics and pesticides in milk.

Kansas—The March 14 meeting had as its speaker H. C. OLSON, Dairy Department, Oklahoma State University, Stillwater. Dr. Olson discussed the topic: Tests for Sanitation Efficiencies in Dairy Plants.

Massachusetts—The March 7 meeting featured a talk by D. H. JACOBSEN, Research Director, American Dairy Association, reporting on the progress of some of the research projects sponsored by A.D.A.

At the Jan. 25 meeting, FRANKLIN BARBER, National Dairy Laboratories, spoke on new developments in testing for the bacteriological quality of dairy products. He emphasized the importance of tests for specific types of organisms and tests for bacterial inhibitors.

Metropolitan—Gasner's Restaurant, New York City, was the locale of the group's March 8 cocktail-dinner-meeting. P. A. ROHDE, Director, Technical Services, Baltimore Biological Laboratory, Inc., spoke on FDA Test for Penicillin in Milk. New members: LEE CHANNING, Chesterfield Farms, Inc.; W. G. MEYER, Hygrade Milk & Cream Company; E. M. GROPEN, Breakstone Foods.

North Carolina—H. B. HENDERSON, head, Dairy Department, University of Georgia, Athens—chairman of the special A.D.S.A. committee which prepared the new Dairy Industry Plant Training Manual—spoke at the March 9 meeting on the ways whereby the Manual could be used to advantage in any plant. Since its publication recently, the Manual has received many favorable comments from men in industry, as to its value.

Ohio—J. J. MURPHY, Sales Manager, Cherry-Burrell Corporation, Cedar Rapids, Iowa, spoke on Automation in Dairy Operations at the March meeting of the four Ohio Dairy Technology Societies—Maumee Valley, Northeastern Ohio, Central Ohio, and Cincinnati.

Tri-Cities—At the monthly dinner meeting on March 8, CARL HOTTENSTEIN, American Dairy Company, Evansville, Indiana, spoke on his experiences in India. Mr. Hottenstein had attended a Trade Fair in India sponsored by the U. S. Department of Commerce, and was selected by the USDA as a representative of Dairy Society International. The purpose of his attendance was to promote the consumption of dairy products in India.

Tri-State—Subject for the February 24 meeting was What's Ahead in the Milk Industry? D. V. JOSEPHSON, Head, Department of Dairy Science, The Pennsylvania State University, covered the trends developing in the industry today that will be the methods and procedures of tomorrow.

March 23 meeting was to feature the subject How Management Can Reduce Costs, with ROGER SHARKEY, Manager, Pure-Pak Sales Engineering Department, Ex-Cell-O Corporation, Detroit, as speaker.

Utah—Fourth Annual Meeting was held March 1 at Utah State University. H. A. MORRIS, University of Minnesota, spoke on the subject Why Not Standardize M.S.N.F. in Milk?

New officers for 1960-61 are: president—LYLE FORD, Bacto-Chemical Dairy & Food Lab-

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...until we get good cow milking.

we'll never get GOOD COW MILKING

...without milking conditions that enable dairymen to take pride in their job.

we'll never put PRIDE back in DAIRY FARMING

...without a milking setup that's built for the man ...as well as for the milk and the cow.

Unless the cow milker can take pride in his job, no dairy farm or milk plant can count on the kind of milk we all want for our children.

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oratory, Salt Lake City; Vice-President—Estle Smith, Safeway Stores Milk Department, Salt Lake City; Secretary-Treasurer—P. B. Larsen, Department of Dairy Industry, Utah State University, Logan; and Directors: Ancil Winger, Beatrice Foods Company, Orem; Reed Ferris, Nelson Ricks Creamery Company, Salt Lake City; Don Brighton, Klenzade Products, Inc., Salt Lake City.

Western Michigan—March 21 meeting was to be held at Finger's Cafe. Guest speaker: T. I. Hedrick, Dairy Department, Michigan State University, on the topic: Experience with Ultra High Temperatures in Milk and Other Dairy Product Processing.

LETTER TO THE EDITOR

The recently instigated policy of publishing interpretive summaries of scientific papers appearing in the Journal of Dairy Science is one which I believe is not in the best interest of the Journal or the Association. The Interpretive Summary presumably widens the appeal of the Journal to persons in the dairy industry who are unable to comprehend the original articles. The question becomes one of whether or not it should be the function of the Journal of Dairy Science to provide a popularized version of each scientific paper.

The contributions of the American Dairy Science Association to the development of the dairy industry have been largely in the dissemination of scientific facts on which technological advances could be made. The Journal of Dairy Science, as official organ of the Association, should be a scientific publication of sufficient merit and stature to attract all scientific papers relevant to dairy production and technology. Popularization to any degree probably discourages the submission of manuscripts on fundamental research. Members who now publish many articles of basic scientific merit pertinent to the dairy industry should be encouraged to publish in the Journal of Dairy Science. The requirement that authors submit interpretive summaries along with their scientific papers is an additional burden that tends to discourage rather than encourage publication in the Journal. Numerous trade journals provide interpretive reports of scientific studies that lend themselves to practical applications. There appears to be no reason why the Journal of Dairy Science should shift into this area.

Undoubtedly, financial considerations were the basis for the change to a popular presentation in the Journal. I question whether a sufficient increase in advertising and circulation will result to offset the increased cost of publication resulting from the inclusion of "Interpretive Summaries."

R. E. Brown,
Department of Dairy Science,
University of Illinois, Urbana

STUDENT CHAPTER NEWS

J. E. Johnston, Editor
A Section Devoted to News of Student Members

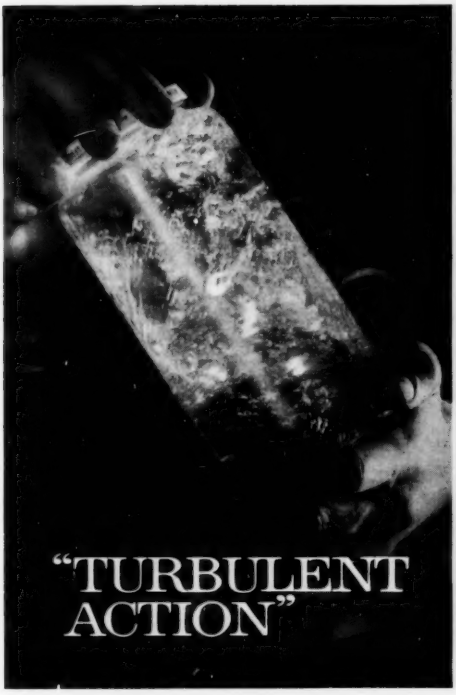
Organization of Student Branch

Plans for the organizational meeting of the Student Branch to be held during the A.D.S.A. Annual meetings in Utah are proceeding rapidly. Paul Reaves of Virginia Polytechnic Institute and his committee, consisting of J. B. Mickle of Oklahoma, J. W. Williams of Minnesota, and A. W. Rudnick of Kentucky, have planned a program which should be of interest to all Student Affiliate members. There will be a series of three meetings covering the following points:

1. To deal with the organization of A.D.S.A. and the place of the Student Branch Section; the purpose of the Student Branch; the proposed organization plan, followed by luncheon with certain A.D.S.A. members.
2. To include report of committees; discussion of activities of local chapters; and discussion of type of program for the 1961 meeting.
3. A review of the Student Chapter exhibits by the judges; election of officers for 1960-61; committee reports, etc.

Dr. Mickle, with the help of interested students and other A.D.S.A. members, has worked out some needed changes to the constitution and by-laws which were approved by the chapters a year or two ago. Each chapter should have a copy of the revised constitution for review prior to the convention.

Nomination of officers. The election of officers for the 1960-61 year is to be an important phase of the convention. Each chapter may nominate one or more members for an office. These nominations should be made by mail and should be in the hands of Professor Reaves by May 10, 1960. Each nomination should be accompanied by one-page description of the qualifications of the nominee. A nominating committee will screen the nominations and present a slate of officers to the section during the



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These action photos dramatize the difference in cleaning power between ordinary in-place *pressure* systems and De Laval's "Turbulent Action" *vacuum* method.

Bottle at left is *half-filled* with water. See the turbulence when it's shaken? This is how De Laval *vacuum* cleaning works. It *pulls* water through lines at up to 35 mph with great turbulence to scour away fat and milk deposits.

Bottle at right is *filled* with water. When shaken, no turbulence occurs. That's how *pressure* systems work. They fill lines with water then *push* it through at only 4 mph without turbulence. "Turbulent Action" insures truly sanitary lines, lowest bacteria counts.

What's more, De Laval's Automatic Washer uses 60% *less* water, *less* electricity, 60% *less* detergent. With *pressure* a dairyman would use 78,840 gallons of water a year for a 50-cow herd . . . only 32,850 gallons with De Laval *vacuum*. And only 228 lbs. detergent as against 547 lbs. with pressure.

MORE SANITARY ADVANTAGES—Unlike all other automatic CIP systems, only De Laval . . .

- *Never* re-circulates rinse water — either in the pre-rinse or clean rinse stage.
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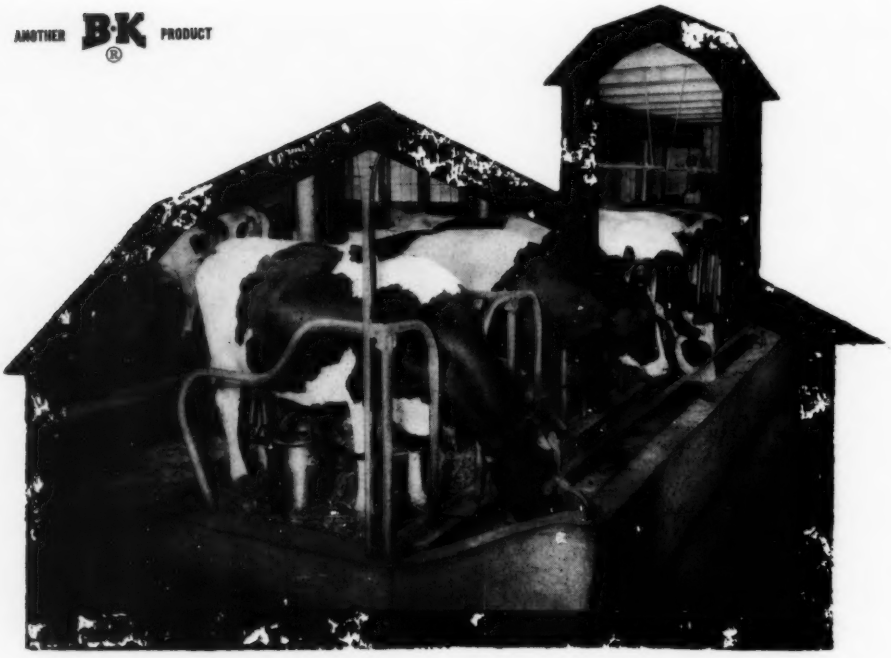

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PENNSAN's outstanding properties have been established by extensive lab tests and highly successful dairy farm use. (Test results on request.) These all demonstrate that PENNSAN:

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convention. The qualifications of nominees are as follows:

1. Nominee must be a student affiliate or regular member of A.D.S.A.
2. He must be an undergraduate or graduate student who will be in school during the 1960-61 school year.
3. He must indicate that he has the interest and the time available to carry out his duties.
4. He must be in attendance at the organizational meeting and plan on attending the 1961 meeting to be held in Wisconsin.
5. Nomination should be approved by an officer of the chapter and either the faculty advisor or department head.

Many chapters have already indicated their intention of sending delegates to this first Annual convention of the Student Branch. Chapters which are unable to send one of their regular members should designate a faculty member to represent them.

An indication of the plans of chapters to send delegates would be appreciated by the organizational committee. Comments on the convention and nominations should be mailed to: Professor Paul M. Reaves, Department of Dairy Science, Virginia Polytechnic Institute, Blacksburg, Virginia.

Minnesota Club News

A milk-vending machine has been placed in the Student Room of the Dairy Industries Building. It is hoped that this machine will publicize the Minnesota Dairy Science Club, in addition to promoting the sale of dairy products.

The expert livestock, poultry, and products judges on the St. Paul Campus were given an opportunity to achieve fame and glory during the weekends between January 23 and February 6 by participating in the Collegiate Winter Judging Contests. A number of club members took part in the organization and preparation of this year's event, as well as in the judging. GERALD STRANDLUND won first-place honors in Reasons, as well as second place over-all in dairy cattle. SHELDON ERICKSON won poultry judging and placed second in the total over-all contest.

On February 9, through the cooperation of the professional clubs on campus, the Ag Stag was attended by 325 students and faculty. This is a stag dinner at which the awards for the Winter Judging Contests are presented. This year the Dairy Husbandry Department was quite honored to have W. E. PETERSEN as master of ceremonies.

The Minnesota Dairy Science Club undertook one major fund-raising project this quarter; namely, serving meals to Farm and Home Week guests. Despite the fact that this year's attendance was very poor compared with pre-

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IOSAN is a patented germicidal cleaner that saves time and labor by replacing two or more single-action products. It quickly cleans and sanitizes bulk tanks and other equipment. Also reduces hot water bills because it is used in tap or lukewarm water.

"Tatties" on milkstone. Iosan "tatties" on hard-to-remove or overlooked accumulations of milkstone with a telltale yellowish-brown stain that is easy to remove. Reduces bacteria counts to consistent lows, leaves equipment sparkling clean.

Kills Mastitis Organisms. Iosan kills streptococcus, pseudomonas, E. Coli, staphylococcus and other organisms that cause and spread Mastitis. Its "Tamed-Iodine" killing power has been substantiated by laboratory tests that meet hospital standards. Iosan provides safe, low cost protection when washing udders and dipping teats. For a free demonstration contact your regular supplier or Lazarus Laboratories Inc., 42-16 West St., Long Island City 1, N. Y.

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By using the Dairy Laboratories system of "Numbered Blends" you're insured complete success in every batch. Highest quality fresh culture *every week* keeps aroma, body and flavor uniform in all fermented milk products.

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vious years, the project was financially successful. The success is attributed in no small part to the desirable reputation the club has built up with its meals. The profit will be used to send a delegate to the A.D.S.A. annual meeting and to purchase awards for the Winter Judging Contests.

Programs presented for the Winter Quarter were high-lighted by an informal panel discussion concerning the problems in the dairy industry. The panel was composed of E. BERGERSEN of Fairmont Foods and MR. STUCKY of Sanitary Farm Dairies. Other programs included a discussion of the problems of the student placement service by P. S. ANDERSON, and an explanation of the publicity efforts of the Minnesota Dairy Industries Association by Minnesota's Princess Kay, BETTY JAX.

Princess Kay will again be the guest of the Minnesota Dairy Science Club on March 7, when she will greet guests at the Winter Quarter Milk Hour. Because of the encouraging success of hot chocolate at the Fall Quarter Milk Hour, the committee is planning to serve only a minimum of regular milk, relying mainly on hot chocolate. A cow equipped with sandwich boards will be escorted around the campus to aid in publicizing the event.

In accordance with the proposed constitution of the National Student Branch of the American Dairy Science Association, the club has named three delegates to the annual meeting. The official delegates are DALE DINGER, DICK SCHMANSKI, and JOHN HEALY.

WANTED

SIRE ANALYST

New England Selective Breeding Assn.

Position entails analysis and evaluation of sires currently used and working with sire committees in selecting sires for future use. Must have advanced training in animal husbandry and genetics. Experience with dairy cattle breeding and judging or as a sire analyst will weigh heavily in selection.

Contact

NESBA, Newton Road
Woodbridge 15, Conn.

Auburn University Dairy Science Club Entertains American Dairy Princess

The annual social of the Auburn Dairy Science Club was held February 16, 1960. The social was sponsored partly by the Auburn Dairy Alumni Association. High light of the meeting was the appearance and talk of the American Dairy Princess, MARY SUE HODGE of Snover, Michigan. Special guests also attending were Alabama Dairy Princess BARBARA ALYS BAILEY and Alternate Alabama Princess SARAH MATTHEWS.

The Dairy Science Club had as guests a number of general agriculture students who have backgrounds in dairying.

Kansas Chapter Election of Officers

The Kansas State University Dairy Science Club had an election of officers during the regular meeting on January 12, 1960. The following list of officers are those which were elected to hold office for the spring semester:

President—BYRON WARTA

Vice-President—RICHARD ROSENHAGEN

Secretary—JOHN CARLIN

Treasurer—KENNETH MCCOSH

Reporter—DEAN VINCENT

Parliamentarian—CARROL CONYAC

Program Chairmen:

Chairman—JIM SWIERCINSKY
Subchairman—DON FINICAL
Asst. Dairy Student Editor—JOE MINK

Penn State Club News

New officers for the spring semester were recently installed. The new officers are: President, RON DIETZ; Vice President, AL NORMAN; Secretary, LINDA METZGAR; and Treasurer, LARRY BEAVER. Faculty advisors are W. T. O'DELL, Manufacturing, and J. R. NICHOLS, Production.

A number of committee chairmen were appointed to direct the club's activities for the coming year. Included were MARGARET DITRICH, chairwoman scrapbook committee; BRONNIE LANE, Publicity; BOB WARNEKE, Showcase; JOE KUNSMAN, FRANK MADDEN, and STEVE WITMER, student council representatives.

The Annual Penn State Dairy Exposition has been scheduled for May 7, 1960. This is one of the big events which the Dairy Science Club sponsors annually. It includes a dairy

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A Remarkable NEW Product . . . with Even More Remarkable Properties

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WHY IS Chlorinated KLEER-MOR DIFFERENT?

It's chlorinated with exclusive organic chlorine
Contains no out-dated tri-sodium phosphate
It sparkles . . . crystal clear . . . instantly soluble
Exceptional water conditioner — will handle all water conditions
Foam-stabilized with high wetting speed
Extraordinary detergency, yet moderately alkaline for hand use

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The publication of the Penn State Dairyman is an integral part of the entire Exposition and serves as a permanent record of the many events and activities of the club. Every dairy science student is included in some way, as well as the dairy staff and others who contribute to the club's functions. One of the most responsible positions in the club is that of editor of the Dairyman. This year it is in the very capable hands of DEAN GIRTON, a senior in dairy manufacturing. His staff includes RAE NEWLIN, associate editor; FRED FOREMAN, business manager; and GEORGE SHOOK, patron manager.

Students in Southern Section Plan Organization

The Clemson College Student Branch of the American Dairy Science Association, with an objective of strengthening the ties between the individual student chapters and the national chapter, conceived the idea of forming a regional student branch of A.D.S.A. Letters postulating such an idea were mailed to 12 college chapters in the southern states. The responses to these letters were encouraging, so the 12 chapters were invited to have delegates at the regional meeting of the Southern Section of A.D.S.A. in Birmingham on February 1-3, 1960.

On February 2, 1960, student delegates representing five chapters were called to order for the purpose of considering the possible formation of a Section Student Branch of the American Dairy Science Association. JOHN SWARTZFAGER, immediate past president of the Clemson Chapter, presided. The chapters in attendance were Virginia Polytechnic Institute, Auburn University, Clemson College, University of Georgia, and Mississippi State University. Faculty advisors present were from V.P.I., Clemson, Georgia, Auburn, Tennessee, L.S.U., Oklahoma, and Mississippi. The morning meeting was devoted to a discussion of the advantages and disadvantages of organizing a sectional branch of the A.D.S.A. The point of most concern was the possible effect it would have on strengthening the national organization of student chapters.

After a decision was made to form a temporary organization of the southern chapters, the afternoon session was devoted to such a task. Officers were elected from the delegates in attendance. By approval of the group, it was decided to elect the chairman and corresponding secretary from the same school. W. L. WEBB and K. BOUTWELL, JR. of Mississippi State University, were elected to these positions, respectively. JOE LIVEWEAVER of V.P.I. was elected vice chairman and charged with the responsibility of presenting the views of the regional organization at the national meeting to be held in Utah. WAYNE BOONE of Clemson College was elected recording secretary. J. T. CARDWELL, Mississippi, J. T. LAZAR, Clemson, and PAUL REAVES, V.P.I., were elected to a committee of faculty advisors, with Dr. Cardwell as chairman.

During the afternoon session several of the faculty members of the Student Affiliate Committee of A.D.S.A. spoke to the group about the national organization. A. C. FAY, President of the A.D.S.A., met with the students and commended them for their interest and activities in the dairy industry.

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In formulating plans for the sectional organization, the student group agreed to adopt the constitution of the national student organization and to modify it to meet its needs. Because the present organization is a temporary one, invitations will be forwarded to all chapters in the southern states for charter membership. The next meeting will be held in February, 1961, at Jackson, Mississippi.

The purpose of organizing the Southern Student Section of A.D.S.A. is to unify the clubs, strengthen the individual clubs by sharing ideas, strengthen the national organization, form a connecting link between the individual clubs and the national organization, and promote pride in the dairy industry.

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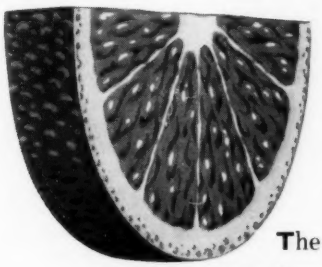

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BOVINE LEPTOSPIROSIS. A REVIEW [1]

L. E. HANSON
Department of Veterinary Pathology and Hygiene,
University of Illinois, Urbana

SUMMARY

Bovine leptospirosis is a disease of cattle caused by infection with motile filamentous bacteria belonging to the genus Leptospira. *Leptospira pomona* is the most prevalent species involving both cattle and swine in the United States.

Leptospires are readily destroyed by heat, desiccation, and most chemicals, but can survive for extended periods in stream and pond water. Moving streams provide a ready mode for dissemination, since skin contacts with urine-contaminated water apparently result in the most frequent route of infection.

Fever, anemia, mastitis, and abortion are the most characteristic signs of leptospirosis in infected cattle. The kidney is affected most consistently, with the development of an interstitial nephritis.

Laboratory diagnoses of leptospirosis may be accomplished by isolation of the organism in artificial media and laboratory animals, by demonstration of organisms in kidney and liver tissues with silver stains, or by demonstration of antibodies with various serological tests. Most diagnostic laboratories routinely perform serological tests.

Although some antibiotics aid in the control of leptospirosis, treatment to be effective, must be started early in the course of the disease. Vaccination is widely practiced in all areas of the United States. Experimental studies with the various vaccines available demonstrate that a definite but not complete or durable protection is provided by these vaccines.

Leptospirosis was first recognized in Russia as a disease of cattle in 1935 (46). Nine years later, Jungherr (39) described the disease and demonstrated the organism in cattle tissues in the United States. Baker and Little (5) isolated the first organisms from American cattle 4 yr. later.

Bovine leptospirosis is now recognized as one of the major cattle diseases of the United States. The disease was estimated to be responsible for an annual loss to the cattle industry of over $100,000,000 in 1954 by the U. S. Department of Agriculture (68). Obvious losses result from deaths, abortions, and decreased milk production. Less obvious losses occur in weak calves which gain weight very slowly.

CAUSATIVE AGENTS

The spirochetes responsible for leptospirosis are highly motile, filamentous organisms which appear to be beaded, due to their tightly twisted spiral shape. An axial filament runs lengthwise through the organism, providing some rigidity. Both ends of the filament are usually bent. A characteristic type of motility is produced by the spinning of the filament along its long axis. The numerous species of Leptospira can not be distinguished on the basis of morphology. The metabolic reactions frequently used to classify most bacteria are of no value, since leptospires fail to utilize the common nutrients of test media (32, 61).

Classification is, therefore, based on variations in antigenic structures as determined by the agglutination-lysis test. When placed in contact with the

[1] This paper was not intended to be a complete review on Leptospirosis, but it includes the pertinent literature on pathogenesis, causative agents, symptoms, diagnosis, treatment, and control.

homologous serum, leptospires are either agglutinated or lysed. The presence of 10% or more of the serum titer following adsorption with the heterologous serum strain indicates that the strain belongs to a different species (78).

More than 50 serotypes or species have been recognized at the present time (3). In the United States, five species either have been isolated from cattle tissue or their antibodies detected in cattle serum. *Leptospira pomona* is the most widespread species in the United States and is responsible for the major losses due to leptospirosis. *Leptospira canicola* was isolated from a two-day-old calf in Alabama (67) and was incriminated in another case (75, 77). Three other species, *Leptospira sejroe, Leptospira grippotyphosa,* and *Leptospira icterohaemorrhagiae* have been detected serologically, but their isolation has not been reported from cattle.

L. pomona was first isolated in Australia from a person with seven-day fever in 1937 (16), and later from swine in 1939 (38) and from cattle in 1949 (65). In addition, this organism can cause disease in sheep(6), goats (48), and horses (60). Although swine appear to be the primary carriers (72), transmission may occur directly between cattle and also apparently from cattle to sheep (6). Recently, McKeever *et al.* (45) isolated *L. pomona* from raccoons, skunks, and wildcats in Georgia, and Borg-Petersen (7) from field rodents in Denmark. The presence of this organism in these animals suggests that they may be a reservoir which could play an important role in initiating outbreaks in domestic animals. Antibodies have been detected in the sera of an appreciable number of deer in various areas of the United States (24, 62, 76), but the possible role of deer as disseminators has not yet been determined.

Several workers (26, 58) have detected significant levels of *L. grippotyphosa* and *L. sejroe* antibodies in the serum of American cattle. These species have not been reported from cattle. Although signs of leptospirosis have usually not been associated with the presence of *L. sejroe* serum antibodies (26), abortion, reduced milk flow, and loss in weight were associated with the development of *L. sejroe* and *L. grippotyphosa* antibodies in cattle in Illinois (4). *L. grippotyphosa,* however, has been isolated from the raccoon in Georgia (45).

The members of the genus Leptospira can be cultivated in liquid, semisolid, and solid media. Cultivation requires a medium enriched with 10 to 15% mammalian serum. Rabbit serum is most commonly used. Various liquid media have been developed for the propagation of Leptospira (13, 64). Semisolid media are used in some laboratories for maintenance of cultures, since the organisms multiply more slowly than in the liquid cultures, making fewer transfers necessary. The organisms can be cultivated to a limited degree on solid media (19). The colonies grown on solid media show some variation in morphology. Temperatures of 28 to 29° C. appear to be optimum for growth, while temperatures above 30° C. cause inactivation and death (14). Pasteurization temperatures, therefore, render milk safe for human consumption.

Although leptospires are easily destroyed by heat, sunlight, desiccation, chemical disinfectants, and strong acids and bases, they can survive in relatively wide ranges of environmental conditions. A moist environment with moderate

temperatures is best. Considerable variation in survival time has been reported in many types of environment. Surface waters in streams and ponds apparently provide the most common media of dissemination and, therefore, have been studied extensively. Van Thiel (73, 74) detected virulent organisms for as long as 22 days in surface water. Leptospires were detected for seven days in nonsterile river water and for 94 days in Seitz-filtered river water by mouse inoculation (50). Okazaki and Ringen (50) detected live organisms for as long as 183 days in water-saturated soil. The pH of the water is important as demonstrated by Chang *et al.* (15). They found that *L. icterohemorrhagiae* would survive in fresh water for only 28 hr. at pH 5, but would survive for as long as 30 days at pH 7. Its survival time was reduced to 18 to 20 hr. in salt water (15).

Kirschner and McGuire (41) observed that *Leptospira hyos, L. icterohaemorrhagiae,* and *L. pomona* were lysed by milk from cows, goats, and man. The antileptospiral effect of the milk was not reduced by storage, pasteurization, or heating for 5 min. at 80° C.

The survival time of leptospires in the urine of animals and man has been studied by various workers. Davidson and Smith (21) found that *L. icterohaemorrhagiae* could survive as long as 6 hr. in the urine from persons not exposed to Leptospira. Van der Hoeden (69) found specific agglutinins and lysins in the urine of infected men, dogs, and rats.

PATHOGENESIS

Leptospires may enter the body of an animal by several routes. Penetration of the abraded skin of feet and legs in wading of streams, field ponds, and marshy areas is probably the most frequent portal of entry in cattle. The organisms can also enter the mucous membranes of the eyes, nose, and the mouth.

The organisms multiply quite rapidly in the blood stream after penetration of the skin. The incubation period is three to seven days. Elevation of body temperature varying from 103 to 107° C. is usually the first detectable sign of leptospirosis. Organisms may be isolated from the blood of cattle during the period of demonstrable fever and until antibodies are detectable in the blood. Reinhard and Hadlow (53), using the agglutination-lysis test, detected antibodies in cattle as early as four days, but in some animals antibodies were not detected until 18 days following artificial exposure.

Leptospires may be released in various excretions of the body, but the most important avenue is the urine. Leptospira are most consistently present in kidney tissue; they enter the kidney early in the infection and later localize in the renal tubules. They are released into the tubules and carried through the urinary bladder and out of the body with voided urine. Most workers have found relatively few leptospires in cattle urine and have had difficulty isolating the organisms. However, Gillespie *et al.* (31) observed large numbers of *L. pomona* in the urine of cattle. In the febrile stages of the disease, organisms may be isolated from the blood and occasionally have been detected in milk (5). Several isolations have been made from aborted fetuses (20, 52), however, most isolation attempts have failed.

SIGNS

The onset of fever, which is often accompanied by anorexia and inappetence, may be followed within a day by anemia, albuminuria, hemoglobinemia, and a sudden decrease in milk flow. The milk secreted is usually viscous and yellowish, but no marked swelling of the udder is detectable. In some animals, extensive hemolysis occurs, causing the milk to appear pink and the urine dark red. Icterus of most body tissues is present in severe cases. Deaths may occasionally occur. Although cattle of all ages are susceptible, the disease is usually most severe in calves and feeder cattle. Abortions may occur in pregnant animals, usually taking place ten to 16 days following acute signs (23, 43, 47). Ferguson *et al.* (23) suggested that toxemia rather than direct infection of the fetuses may be responsible for abortions, since many workers have failed to isolate organisms from the aborted fetuses. On the other hand, Fennestad *et al.* (22) demonstrated leptospires in a number of fetuses and believed that invasion of the fetus by leptospires is the factor responsible for abortion in cattle. Bridges (8) suggested that failure to isolate leptospires is due to death and disintegration of organisms after the fetus dies. TePunga and Bishop (66) suggested that interruptions of the fetal maternal cotyledon junction may be caused by localized lesions. Morter *et al.* (49) stated that they found lesions in the cotyledon which supported this theory. Also, Morse (47) reported that retention of fetal membranes following abortion occurred in 20% of the outbreaks in Wisconsin.

LESIONS

The lesions in bovine leptospirosis are primarily confined to the kidneys. In the acute stage, petechiae are often present on the surface of the kidneys and may be accompanied by hemosiderin deposits. Small white foci are frequently observed on the surface of kidneys following the acute stages of leptospirosis (17, 33, 53). Microscopic examination of affected kidney tissue reveals an interstitial nephritis. Leptospires can often be demonstrated in affected tubules when silver stains are used (8).

The liver tissue may be markedly yellow and congested in severe cases, and petechiae and icterus may be observed in various mucous membranes.

DIAGNOSIS

The clinical signs of bovine leptospirosis may be highly suggestive, but are not pathognomonic of the disease. Due to the varied signs produced, and the frequent absence of some of them in many outbreaks, serological and isolation techniques are required for a definite diagnosis.

Serological diagnosis is based on the detection of antibodies primarily in the blood, although other body fluids are occasionally used. The agglutination-lysis test is apparently the most accurate test, although it is somewhat cumbersome, since live organisms must be used for the antigen and the test has to be read under a darkfield microscope. Several plate agglutination tests which have been developed are widely used by laboratories and practicing veterinarians in screen-

ing sera for positive reactions. However, the plate agglutination tests are less sensitive than the agglutination-lysis test and do not measure the antibody concentration. A capillary tube agglutination test using a formalin-killed antigen is also used and has the advantage over the plate agglutination-lysis test of measurement of antibody concentration. Van der Hoeden (71) has developed a milk agglutination test. At present, some laboratories use the rapid plate test for screening samples, and either an agglutination-lysis test or a capillary test to determine the antibody concentration (63). All of these tests are species specific, which necessitates repetition of each of the antigens. Galton *et al.* (27) recently developed combination antigens, each of which contains a group of several species to be used for screening purposes. Once a group has been identified with these combined antigens, single species antigens can be used for further identification.

A complement-fixation test is also used in some cases, but the complement-fixing antibodies do not appear as early and they do not persist as long as the agglutination-lysis antibodies (81). Cox (18) has developed a hemolytic test which appears to be genus specific rather than species specific.

Detection of antibodies alone does not constitute a positive diagnosis. Vaccination of cattle with *L. pomona* bacterin can also cause formation of antibodies which are detectable by serological tests. Positive reactions at titers of 1:100 or more by the agglutination-lysis or capillary tube test are usually considered significant. Since titers persist for years, the present disease may or may not be associated with *L. pomona*. Therefore, detectable antibodies are important only if their titer rises within a period of several weeks following clinical evidence of the disease. Correlation of an increase in antibody level during a short period of time with the presence of clinical signs provided a reliable method of leptospirosis diagnosis.

Cultures can be made from infected tissues, urine, milk, and surface water, either directly in artificial media or indirectly by inoculation of laboratory animals, followed by isolation in artificial media. Guinea pigs, hamsters, and young chickens have been used extensively in *L. pomona* studies (25, 37, 57). Some workers consider chinchillas (59) and gerbiles (70) to be more satisfactory laboratory hosts than guinea pigs.

TREATMENT

Antibiotics have been used extensively in the treatment of leptospirosis in cattle, man, swine, and dogs. Early studies indicate that penicillin is inhibitory but apparently primarily bacteriostatic in action (2, 12). Other antibiotics which have shown to have an inhibitory effect upon Leptospira are oxytetracycline, streptomycin, chlortetracycline, chloramphenicol, erythromycin, and tetracycline hydrochloride (10, 34, 42, 55, 56, 79). Early medication is of primary importance, since the drugs are more effective early in the development of infection. Medication must be started before extensive kidney damage occurs. Administration of antibiotics in feed has been reported to decrease signs in infected herds (23, 36). Various sulfonamides have been used with very little success.

Other drugs have proved to be ineffective. Antiserum has been used in man with some success, but is not economically practical in animals.

The levels of antibiotics used in semen extenders appear to be sufficient to eliminate the semen used in artificial insemination as a mode of spread in bovine leptospirosis (11, 54).

VACCINATION

Vaccination of cattle with bacterin is widely practiced at the present time in the United States. Although indifferent results were obtained in early studies (51), recent work has been more encouraging (28, 29, 40). The vaccines most extensively used are cultures inactivated with formalin, antibiotics, drying, and thimerosal and are standardized to a specific concentration (9). These vaccines produce varied resistance to *L. pomona* for six or more months (28, 30, 40). Calves under three months of age from dams not carrying antibodies, as well as from serologically positive dams, apparently do not respond to bacterins; therefore, most manufacturers advise against vaccinating calves until they are at least 3 months old (29).

York and Baker (80) reported that a chicken embryo bacterin inactivated by repeated freezing and thawing produced good protection. Reinhard and Hadlow (53) found that an egg-attenuated vaccine protected experimental cattle for 17 mo. Hoag and Bell (35) used an acid-heat-extracted culture of *L. pomona*, which provided protection for 1 and 2 months with little stimulation of agglutination-lysis antibodies. McDonald and Ridge (44) reported that vaccination of cows in late pregnancy provided protection of calves for the first few months of life. Alexander *et al.* (1) reported that significant antigenic variations exist between the types or strains of *L. pomona* which may be great enough to affect the degree of resistance produced by a vaccine prepared from a single strain.

At present, the commercially available bacterins for cattle are prepared from *L. pomona* and will not stimulate adequate resistance to the other species of Leptospira which have been detected in cattle in the United States. The duration of immunity following vaccination has not been adequately investigated. Only a large-scale, controlled vaccination study can demonstrate the actual value of vaccination as a control measure against bovine leptospirosis. There appears to be little point in vaccinating cattle having positive serological tests.

Since both treatment and vaccination have limited value, every effort should be made to prevent entrance of leptospirosis in a herd of cattle. Since swine are apparently the primary carriers of *L. pomona* in the United States, it is advisable to keep cattle and swine separated as much as possible.

Leptospirosis serological tests should be conducted on all animals before purchase.

Exposure should be avoided by proper drainage or fencing off of streams open to outside contamination. Feeder cattle of unknown leptospirosis status should not be pastured next to permanent beef or dairy herds.

Cattle with signs of leptospirosis should be isolated immediately, and serological tests should be made immediately and again a week or more later to diagnose the condition.

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IDENTIFICATION OF VOLATILE CARBONYL COMPOUNDS FROM CHEDDAR CHEESE [1]

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SUMMARY

The volatile flavor fraction was isolated from Cheddar cheese by distillation of cheese slurries at reduced pressure and the carbonyl compounds studied as 2,4-dinitrophenylhydrazones.[4] The mixture of DNP-hydrazones was separated and the individual components purified by the column partition chromatography procedures described herein. The DNP-hydrazones of 2-nonanone, 2-heptanone, 2-pentanone, butanone, acetone, 3-methylbutanal, propanal, ethanal, methanal, and 3-methylthiopropanal were identified. Tentative identity of the DNP-hydrazones of 3-hydroxy-2-butanone, and 2,3-butanedione also was obtained. The approximate concentrations of the identified compounds in cheese were determined.

The chemical nature of Cheddar cheese flavor has been investigated for many years. Although a large number of compounds have been reported, disagreement still prevails among researchers concerning the significance of various compounds to the flavor. The importance of the nonvolatile components to Cheddar flavor is recognized and some progress has been made in the study of the fraction (23). Whether similar progress has been made in elucidating the composition of the volatile fraction is still a matter of opinion. Attempts to resolve the mixture have failed, as demonstrated by organoleptic evaluation of synthetic mixtures composed of the identified compounds (6). This suggests that either the missing components are very susceptible to destruction or they occur at concentrations below that of chemical detection. Either may be the case with certain members of the carbonyl family. Several workers have studied these compounds (1, 4, 5, 22), but their significance to flavor is still obscure.

A recent report from this laboratory presented strong indirect evidence that volatile aldehydes are significant contributors to cheese flavors (15). Methional, (3-methylthiopropanal), an aldehyde derived from methionine, was reported to be a particularly important contributor to the flavor. The following study was undertaken in order to obtain further information on the role of volatile carbonyls in Cheddar cheese and to ascertain if the postulated flavor aldehydes actually exist in Cheddar.

Received for publication January 10, 1959.

[1] Scientific Article No. A-741, Contribution No. 2988, of the Maryland Agricultural Experiment Station, Dairy Department. Presented at 53rd Annual Meeting, American Dairy Science Association, Raleigh, North Carolina, June 19, 1958.

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[4] 2,-4-Dinitrophenyl is hereinafter abbreviated DNP.

MATERIALS

Cheddar cheese. Three 40-lb. blocks of rindless Cheddar were used in the investigation. All cheeses were over 1 yr. old and were judged to be of medium to high quality.

Chloroform. Fisher Certified chloroform was found to contain significant quantities of methanal, ethanal, and acetone. Attempts to completely remove these contaminants were unsuccessful. The amounts present per unit volume of chloroform were determined and accounted for when used in the identification and quantitative studies reported herein.

Nitromethane. Reagent grade.

Hexane. High purity grade hexane (Phillips Petroleum Co.) distilled from KOH pellets. Collect the fraction boiling between 68–70° C.

Equilibrated hexane. Hexane from the above treatment is thoroughly shaken with 2% of its volume of nitromethane in a separatory funnel. The lower nitromethane-rich layer can be re-used for subsequent equilibrations.

Celite. Johns Manville Analytical Filter Aid (Fisher Sci. Co., No. C-211).

Chromatographic tubes. A convenient size tube is 60 cm. by 32 mm. o.d. with a 2-cm. length of 6-mm. o.d. tubing fused to the constricted end.

Waring Blendor. The speed is regulated by a rheostat.

METHODS

Modification of the Kramer-Van Duin column partition chromatography procedure. A number of chromatographic procedures were evaluated in an attempt to find a suitable method for resolving DNP-hydrazone mixtures of carbonyls of the type encountered in Cheddar cheese. The column partition procedure of Kramer and Van Duin (16), using nitromethane on silicic acid as the stationary phase and low boiling petroleum ether as the mobile phase, gave excellent resolving power but caused decomposition of some derivatives (particularly the hydrazone of methional). Decomposition of DNP-hydrazones on columns employing silicic acid has been reported by Forss and Dunstone (9). In addition to the decomposition problem, the use of low boiling petroleum ether caused cracking and channeling of the columns, especially in warm laboratories. Substitution of Celite for silicic acid solved the problem of DNP-hydrazone decomposition, and the use of hexane in place of petroleum ether prevented development of cracks and channels in the packed columns. The procedures as developed in our laboratory were briefly described previously (8) and have been successfully employed in several studies by Patton and co-workers (20, 21). Since complete details have not been published, the procedures finally developed are given here. A similar method has been reported recently by Monty (19).

Preparation of column—Method A. This method was recommended for chromatographing the DNP-hydrazones of the C_5 and longer chain carbonyls. A weighed amount of Celite was placed in the mixing bowl of a Waring Blendor, followed by hexane at the rate of 10 ml. hexane per gram of Celite. The rate of mixing was gradually increased by adjusting the rheostat until the mixture exhibited a distinct rolling action in the Blendor, at which time 1.1 ml. of nitro-

methane per gram of Celite was added to the slurry from a burette and mixing was continued for 10 min. After mixing, the slurry was transferred to a separatory funnel for subsequent addition to the chromatographic tube. The column was prepared by first tamping a small glass wool plug into the constricted end. Then the slurry was allowed to drain from the separatory funnel into the tube. Air pressure (5–10 p.s.i.) was applied between aliquots to compact the column. Upon completing the transfer to the column, the column was packed by air until the solvent layer was approximately 1 cm. above the Celite. It was important that a free solvent layer was present above the slurry at all times during packing, to avoid air pockets. Final packing was accomplished by firmly compressing the Celite mixture with a ramrod (a cork slightly smaller than the inside diameter of the tube, and attached to a metal rod). The solvent used for packing the column was either discarded or used to prepare equilibrated hexane (see Method B).

The DNP-hydrazones were dissolved in a minimum volume of hexane (10 ml. or less) and carefully added to the top of the column. The solution of derivatives was allowed to flow into the column, after which two 10-ml. aliquots of hexane were added to wash it into the partition system. Hexane was then added to the top of the column by means of a syphon and the chromatogram was allowed to develop.

Preparation of column—Method B. The method differs from Method A in that equilibrated hexane was used in all instances instead of hexane, and nitromethane was added to the equilibrated hexane-Celite slurry in the Waring Blendor at the rate of 0.75 ml. per gram of Celite. It was recommended that unknown mixtures be chromatographed by Method B (20 g. Celite) as the first step in fractionation. The fast moving components from Method B can then be concentrated and separated by Method A (60 g. Celite).

Chromatography of known 2,4-dinitrophenylhydrazone mixtures. The DNP-hydrazone derivatives were prepared from authentic carbonyls by the method of Shriner, Fuson, and Curtin (25) and were purified by recrystallizations from ethanol-water solutions until constant melting points were obtained. In several cases, it was necessary to purify derivatives by passing them through the columns described herein. The DNP-hydrazones of the C_6-C_{10} alk-2-enals were prepared and contributed by Forss (10). The *cis* and *trans* isomers of 2-furfural were prepared by Braddock and associates (3).

Mixtures of the DNP-hydrazones listed in Table 1 were chromatographed on Columns A and B. Volumes of effluent were carefully measured, to establish definite relationships between a given volume of effluent and a specific DNP-hydrazone. These were: (1) Threshold volume, which was the volume of mobile phase per gram of Celite required to move a band from the top of the column to the lower end, as evidenced by the first trace of color in the effluent; (2) band volume, which was the volume of mobile phase per gram of Celite needed to elute the band from the lower end of the column.

The threshold volumes presented in Table 1 are the averages of at least three chromatograms. The volume of mobile phase required to move a particular DNP-

TABLE 1

Elution of 2,4-dinitrophenylhydrazones from columns by Methods A and B

2,4-Dinitrophenyl-hydrazone	Average threshold volume[a]	Average band volume[b]	2,4-Dinitrophenyl-hydrazone	Average threshold volume[a]	Average band volume[b]
Method A:	*(ml.)*		Method B:	*(ml.)*	
2-Tridecanone	4.1	0.4	2-Pentanone	13.3	2.5
2-Undecanone	5.1	0.7	2-Methylbutanal	17.0	3.0
n-Dodecanal	5.1	0.5	3-Methylbutanal	17.5	4.0
n-Undecanal	5.9	0.6	n-Pentanal	17.5	4.0
2-Nonanone	6.8	0.8	Butanone	17.9	4.8
n-Decanal	6.9	0.5	2-Methylpropanal	21.2	5.0
2-Octanone	8.0	1.0	n-Butanal	22.6	4.8
n-Nonanal	8.2	0.8	Acetone	30.3	6.5
Dec-2-enal	8.3	1.0	Propanal	30.3	6.8
2-Heptanone	9.9	1.3	But-2-enal	37.0	8.0
n-Octanal	10.2	0.8	*cis*-2-Furfural	44.5	12.0
Non-2-enal	10.3	1.4	Ethanal	47.5	11.5
n-Heptanal	12.2	1.2	Propenal	48.4	10.0
2-Hexanone	12.3	1.3	Methanal	70.9	14.3
Oct-2-enal	12.8	1.5	3-Methylthiopropanal	84.0	25.0
2-Pentanone	14.6	1.4	*trans*-2-Furfural	124.0	50.0
n-Hexanal	15.4	1.5	3-hydroxy-2-butanone	222.0	
Hep-2-enal	16.3	1.8	Reagent	265.0	
2-Methylbutanal	18.9	1.7			
n-Pentanal	19.6	1.7			
3-Methylbutanal	19.6	1.7			
Hex-2-enal	21.0	2.8			
2-Methylpropanal	25.0	11.0			

[a] Volume mobile phase per gram Celite to move band to lower edge of column.
[b] Volume mobile phase per gram Celite to elute band from lower edge of column.

hydrazone through a defined amount of the partition column was highly reproducible and was related to the structure of the derivative. These relationships have been reported by Van Duin (27). Threshold volumes, therefore, can be used as clues in the identification of unknowns, just as R_f values are used in paper chromatography. The data included in Table 1 show that the column methods are efficient for resolving homologous series differing by one carbon atom. A distinct, clear zone always existed between the bands when chromatographing a homologous series and the between-band volumes increased progressively as the chain length of the parent carbonyl decreased. The data further reveal that mixtures containing several classes of carbonyls, such as alkan-2-ones, alkanals, and alk-2-enals, with respective chain lengths of C_n, $C_n + 1$, and $C_n + 2$ are difficult to separate by the above partition system. A mixture of octanone, nonanal, and dec-2-enal illustrates the point. Table 1 shows practically identical threshold volumes for these compounds and a mixture of the three, therefore, would be difficult to resolve. The absorption method of Pippen *et al.* (24), and the paper chromatography procedure of Gaddis and Ellis (11), may be useful for resolving such mixtures. Since the types of carbonyls that may occur in a specific band are limited to one of each class (see above), identification of the compound(s) contained therein is simplified considerably by carrying out initial separations with the partition column.

In separating unknown mixtures, one frequently encounters carbonyls with chain lengths ranging from one to 15 or longer. Method A provides fast separa-

tion of the longer-chain members, but the short-chain members move relatively slow in comparison to Method B. On the other hand, Method B will not give resolution equal to Method A when separating mixtures of DNP-hydrazones of the long-chain carbonyls. Therefore, a combination of both procedures has proved useful for separating unknown mixtures.

Recovery of 2,4-dinitrophenylhydrazones. Agreement of the melting points of DNP-hydrazones before and after passage through the column, coupled with complete recovery of derivatives from the column as evidenced by spectral analysis, was indicative that the derivatives were stable on the nitromethane-hexane-Celite columns.

Preliminary results on the volatiles from Cheddar. The method selected for isolating the volatile carbonyls from Cheddar, while very inefficient, was considered most desirable for this study. It consisted of macerating the cheese into a slurry with cold distilled water followed by distillation of the volatiles at reduced pressure. The greater efficiency of atmospheric distillation was recognized, but excessive heat treatment was considered undesirable in view of the apparent delicate properties of Cheddar aroma. The apparatus described by Day *et al.* (7) was modified by substituting ethanol–dry ice traps for all liquid nitrogen traps, except the last one which was eliminated. Eight liters of the cheese-water slurry, containing 20% cheese solids, was used per distillation, which amounted to distilling approximately 5 lb. of cheese per distillation. The distillation was carried out at 20–30 mm. mercury pressure and was terminated when the wet ice trap (2-liter capacity) was filled. A total of 2.5 liters of distillate was collected, including that of the ethanol–dry ice traps.

Results of organoleptic and qualitative chemical examination of the aqueous distillates from Cheddar samples are worthy of mention at this point. Examination of individual traps in the distillation train for odor revealed that the wet ice and first dry ice traps were cheese-like, but also exhibited a fruity odor, while subsequent dry ice traps possessed a malty-sweet type odor suggestive of methyl sulfide. Treatment of aliquots from the combined distillate of all traps with various reagents gave the following results: 10% NaOH destroyed practically all of the typical odor and an amine type odor developed; addition of dilute H_2SO_4 caused an increase in odor intensity; acidified DNP-hydrazine caused an immediate increase in odor intensity which diminished and became more fruity upon standing. Mercuric chloride caused a slight decrease in the intensity of the sweet-malty type odor. These observations suggest that definite odor arises from fatty acids, while esters, carbonyls, sulfides, amines, and possibly acetal type compounds also may be significant. Since the volatile carbonyls were of particular interest to this investigation, attention was directed to this family of compounds.

Isolation of volatile carbonyls from Cheddar. The procedure followed for isolating and separating the volatile carbonyls from Cheddar is outlined in Figure 1. Forty-pound blocks of Cheddar were distilled (5 lb. per distillation) by the above described method, to obtain a desirable concentration of carbonyls for identification studies. The carbonyls from each distillation were reacted to form DNP-hydrazones by addition of excess reagent and sufficient H_2SO_4 to assure 1.0 *N*

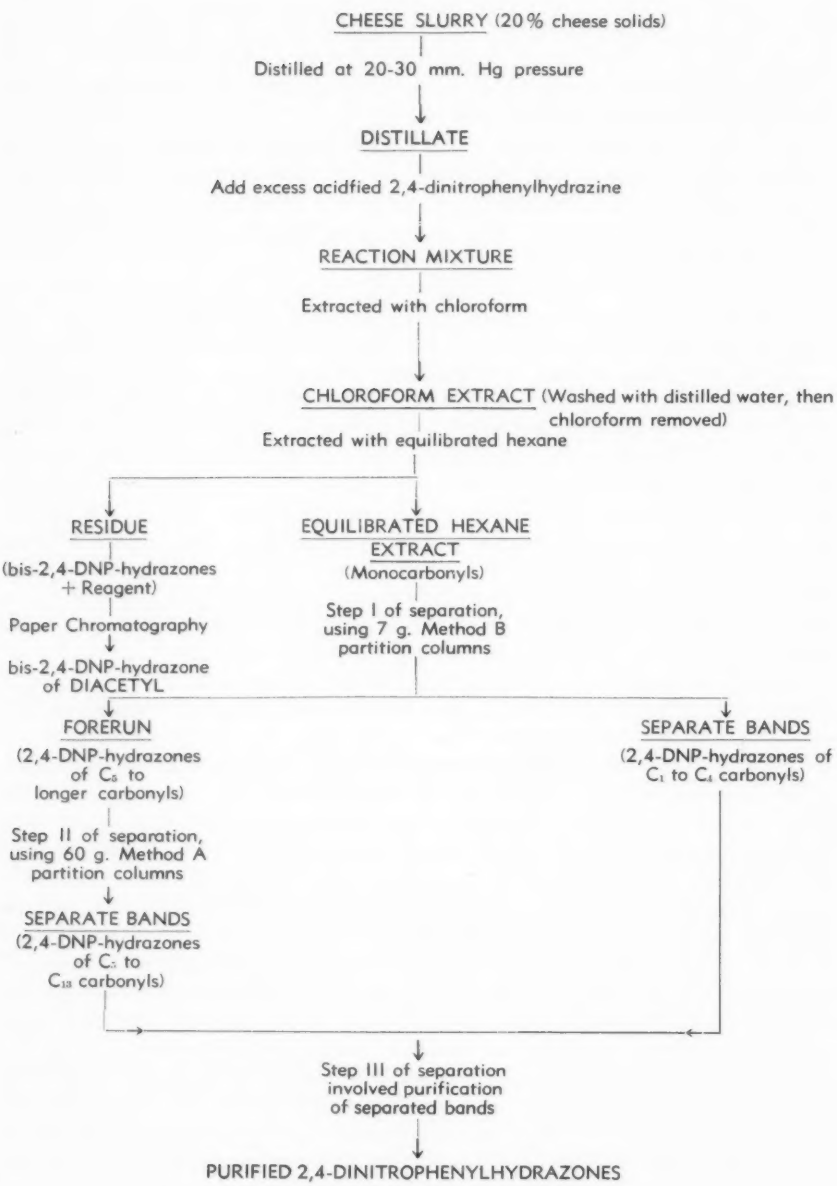
CHEESE SLURRY (20% cheese solids)
Distilled at 20-30 mm. Hg pressure
DISTILLATE
Add excess acidfied 2,4-dinitrophenylhydrazine
REACTION MIXTURE
Extracted with chloroform
CHLOROFORM EXTRACT (Washed with distilled water, then chloroform removed)
Extracted with equilibrated hexane
RESIDUE
(bis-2,4-DNP-hydrazones + Reagent)
Paper Chromatography
bis-2,4-DNP-hydrazone of DIACETYL
EQUILIBRATED HEXANE EXTRACT
(Monocarbonyls)
Step I of separation, using 7 g. Method B partition columns
FORERUN
(2,4-DNP-hydrazones of C5 to longer carbonyls)
Step II of separation, using 60 g. Method A partition columns
SEPARATE BANDS
(2,4-DNP-hydrazones of C5 to C13 carbonyls)
SEPARATE BANDS
(2,4-DNP-hydrazones of C1 to C4 carbonyls)
Step III of separation involved purification of separated bands
PURIFIED 2,4-DINITROPHENYLHYDRAZONES

FIG. 1. Scheme of isolation and separation of volatile carbonyls from Cheddar.

acidity in the reaction mixture. After standing 12 hr., the DNP-hydrazones were exhaustively extracted from the aqueous system with chloroform. The chloroform extract (one liter) was washed twice with distilled water (250 ml. each), to remove residual H_2SO_4, and a dry DNP-hydrazone mixture was finally obtained by evaporation of the chloroform at reduced pressure. A total of eight distillations was required to work up 40 lb. of Cheddar. The DNP-hydrazones resulting from each distillation were recovered by the above method and combined for subsequent separation and identification of individual components of the mixture.

Column chromatography of the 2,4-dinitrophenylhydrazone mixture. The dry DNP-hydrazone mixture, representing the carbonyls recovered from 40 lb. of Cheddar, was exhaustively extracted with small volumes of equilibrated hexane to yield one liter of solution containing the major portion of the monocarbonyl derivatives, and a residue composed of reagent and *bis*-hydrazones (see Figure 1). The complexity of the monocarbonyl mixture contained in the equilibrated hexane solution made it desirable to carry out its separation in three chromatographic steps.

Step I in the procedure involved prefractionation of the mixture into a forerun fraction which contained DNP-hydrazones of C_5 and longer-chain carbonyls, and individual fractions of the C_1 to C_4 carbonyl derivatives. This was accomplished by fractionating 20-ml. aliquots of the equilibrated hexane-DNP-hydrazone solution on 7-g. columns (22-mm. o.d.) prepared by Method B (see Methods). Fifty columns were required and the forerun from all columns was combined, as were individual fractions exhibiting identical threshold volumes. This procedure provided a means for concentrating trace components that would otherwise have remained obscure.

Step II involved separation of the forerun obtained from Step I. The eluting solvent was evaporated from the combined forerun at reduced pressure, the residue was dissolved in a minimum volume of hexane, and 5-ml. aliquots were chromatographed on 60-g. Method A columns.

Step III consisted of rechromatographing various bands from Steps I and II in cases where there was evidence of impurities. The amount of column packing and the column method used depended upon the mobility of the DNP-hydrazone concerned.

The initial fractionation provided in Step I gave a band containing the DNP-hydrazones of formaldehyde and what was subsequently determined to be methional. The mixture was successfully resolved on 20-g. Method B columns in spite of the fact that the hydrazone of methional was barely visible. The total quantity of the methional hydrazone recovered amounted to approximately 0.06 mg.

The slow mobility of the DNP-hydrazone of 3-hydroxy-2-butanone on the chromatographic columns made it impractical to elute the band from the column. It was recovered satisfactorily by first eluting the faster moving bands, then extruding the column from the glass tube and cutting the band out. The derivative was extracted from the Celite with methanol and the extract dried and saved for subsequent studies.

Identification of 2,4-dinitrophenylhydrazones. Following separation of the DNP-hydrazones by column partition chromatography, the eluates were taken to dryness and, where possible, the residues were crystallized from low boiling petroleum ether. Identification of the DNP-hydrazones was dependent upon the following data: Chain lengths were ascertained by column partition and paper chromatography (12). Adsorption spectrophotometry revealed the class of carbonyl. In addition, regeneration of the free carbonyl by the method of Bassette and Day (2) was helpful in determining the type of carbonyl present. Agreement between the melting points of the authentic and the unknown DNP-hydrazone, coupled with the absence of depression in mixture melting points, served as the criterion for conclusive identification.

Determination of approximate concentration of identified carbonyl in Cheddar cheese. Three studies were required to establish the approximate concentration of identified carbonyls in Cheddar. These were: Study 1, determination of the quantity of individual carbonyls recovered from 10 lb. of Cheddar; Study 2, determination of the quantity of individual carbonyls recovered from a control simulated Cheddar system; Study 3, determination of the quantity of each carbonyl recovered from a simulated Cheddar system to which known amounts of carbonyls had been added.

The simulated Cheddar system was prepared by blending washed cottage cheese curd and cream to a composition of 30% SNF and 33% fat. Half of this mixture (10 lb. of solids) was used as the control, while the other half contained known amounts of all identified carbonyls except 3-methylbutanal and 3-hydroxy-2-butanone. For convenience, the distillation apparatus was modified for the recovery studies by elimination of the ethanol–dry ice traps and replacement with four traps containing DNP-hydrazine in 2 N H_2SO_4, and the carbonyls were trapped when bubbled through the reagent. In each of the studies, the resulting DNP-hydrazone mixtures were extracted from the reaction mixtures with chloroform and separated by the previously described procedures. The quantity of each carbonyl recovered in each of the studies was determined spectrophotometrically by measuring optical density at the wave length of maximum absorbance of the DNP-hydrazone, in either methanol or chloroform, and calculating the mg. of free carbonyl by the formula:

$$\frac{A_s \cdot MW \cdot 1000}{A_m \left(\frac{1000}{x}\right)} = \text{mg. carbonyl}$$

where A_s = absorbancy
MW = molecular weight of the carbonyl
A_m = molar absorbancy index (listed in Table 3)
x = volume of solution containing the DNP-hydrazone for A_s measurements.

TABLE 2

Identification of 2,4-dinitrophenylhydrazones of carbonyls from Cheddar Cheese

2,4-DNP-hydrazone	Chromatographic threshold vol. *(ml.)*		Corrected melting points (° C.)			λ maximum[a]
	Unknown	Authentic	Unknown	Authentic	Mixture	mμ unknown
Column Method A						
2-Tridecanone	4.2	4.1	.....	69	.....	362
2-Undecanone	5.0	5.1	.....	63	.....	362
2-Nonanone	6.8	6.8	52.0	54.5	50–52	363
2-Heptanone	10.2	9.9	70–72	71	69–71	363
2-Pentanone	15.0	14.1	143	144	143–144	363
3-Methybutanal	20.0	19.6	121–123	122	120–121	358
Column Method B						
Butanone	19.0	17.9	115	115	114–115	363
Acetone	29.0	30.3	124–126	126	124–126	362
Propanal	31.0	30.3	154	156	153–154	357
Ethanal	47.0	47.5	164–165	166	163–166	354
Methanal	72.0	70.9	164–166	166	163–166	348
3-Methylthio-propanal	83.0	84.0	119	121	119–120	357
3-Hydroxy-2-butanone	212.0	222.0	.....	114	.....	357
2,3-Butanedione[b]	.....	.....	.....	315	.....	393 430

[a] The absorption maxima of the unknowns were in agreement with those of the authentic compounds.

[b] Exhibits two absorption maxima.

TABLE 3

Concentration of individual carbonyls found in Cheddar cheese

2,4-DNP-hydrazone	λ Maximum mμ	Am × 10^{-4}	Approximate concentration of carbonyls found in Cheddar *(mg/kg cheese)*
2-Tridecanone	363	2.27[a]	2.10
2-Undecanone	363	2.27[a]	1.43
2-Nonanone	363	2.25[a]	0.68
2-Heptanone	363	2.25[b]	0.82
2-Pentanone	363	2.18[b]	0.37
3-Methylbutanal	357	2.18[a]	Trace
Butanone	363	2.16[a]	12.50
Acetone	363	2.24[a]	8.50
Ethanal	354	2.22[a]	7.50
Propanal	357	2.25[a]	2.60
Methanal	348	1.90[a]	1.00
3-Methylthiopropanal	357	2.21[c]	0.10
2,3-Butanedione	393	4.25[d]	.....
	430		0.68

[a] Reported by Jones *et al.* (14).

[b] Estimated.

[c] Determined in methanol.

[d] Determined in chloroform.

The absorption maxima used in measuring absorbancy and the molar absorbancy index employed in calculating the quantities of each compound used in the studies are given in Table 3. The approximate concentration of each identified carbonyl was then calculated as follows: After subtracting the quantities of carbonyl isolated in Study 2 (see above) from their respective counterparts isolated in Study 3, the per cent recovery was calculated by the formula:

$$\frac{\text{Quantity carbonyl recovered in Study 3}}{\text{Quantity carbonyl added in Study 3}} \times 100 = \% \text{ recovery}$$

$$\text{Mg. free carbonyl/kg Cheddar} = \frac{\text{Mg. carbonyl isolated from Cheddar}}{\text{Kg. of Cheddar} \left(\frac{\% \text{ recovery}}{100} \right)}.$$

Determination of significance of carbonyls to Cheddar aroma. Known concentrations of carbonyls were added to a simulated Cheddar system and evaluated for taste and aroma, both with and without the presence of fatty acids, lactic acid, glutamic acid, and 3-mercaptopropionic acid. Standard concentrated solutions of each compound were used, which made it possible to vary the amounts added to the synthetic mixture. The synthetic mixtures were evaluated by both experienced and inexperienced judges.

RESULTS AND DISCUSSION

Data showing conclusive identification of ten carbonyls isolated from Cheddar cheese are presented in Table 2. Evidence for tentative identification of 2-tridecanone, 2-undecanone, 3-hydroxy-2-butanone, and 2,3-butanedione is included. The isolation of only minute quantities of undecanone and tridecanone, coupled with inherent problems of obtaining suitable crystals of their DNP-hydrazones for melting-point data, prohibited conclusive identification. However, threshold volumes as well as absorption spectra and regeneration studies strongly suggested their existence in cheese. Although an appreciable quantity of 3-hydroxy-2-butanone was isolated from Cheddar, satisfactory crystals of the DNP-hydrazone were not obtained for melting-point data. As in the above cases, its absorption spectrum and chromatographic behavior were identical to the authentic derivative. The 2,3-butanedione fraction obtained by paper chromatography was tentatively characterized by its absorption spectrum and odor upon regeneration. The *bis*-DNP-hydrazone of this compound exhibits absorption maxima at 393 and 430 mμ. Although these data do not rule out the presence of other α-dicarbonyl derivatives in the same fraction, regeneration of the free carbonyl resulted in a typical 2,3-butanedione odor.

In addition to identification of carbonyls listed in Table 1, the presence of a compound similar in odor to δ-decalactone was observed. This compound was extracted along with the DNP-hydrazones from reacted cheese distillates, and invariably appeared as a distinct fraction on the chromatographic columns with a mobility similar to that of the DNP-hydrazone of 2-heptanone.

The procedure used for isolating the carbonyls from Cheddar was very inefficient and the quantities of free carbonyl per kilogram of Cheddar presented

in Table 3 are, therefore, only approximate. The aroma of the complete carbonyl mixture, when incorporated into a simulated Cheddar system, was detectable but not typically cheese-like. The synthetic mixture considered to be most cheese-like contained the following compounds: the carbonyls at concentration reported in Table 3, except the methional level was increased to 1 p.p.m.; fatty acids at levels of grams/kilograms of medium included 1.6 g. acetic acid, 0.3 g. butyric acid, 0.1 g. caproic acid, 0.26 g. caprylic acid, 0.1 g. capric acid, and 1–2 p.p.m. of 3-mecaptopropionic acid. The aroma of this mixture was the most typical of any evaluated but was still incomplete. Attempts to evaluate the mixture by tasting were unsuccessful. This was attributed to lack of a suitable medium. The numerous nonvolatile proteolytic and lipolytic intermediates occurring in cured Cheddar would undoubtedly improve the system.

The inability to reproduce a complete Cheddar aroma may be attributed to inadequate quantitative data for compounds previously reported, as well as the carbonyls identified in this study. The extremely low yields obtained make it plausible to assume that additional unidentified carbonyls may be a contributing factor. It is also logical to assume that qualitative and quantitative data for other types of compounds have yet to be established. The presumptive evidence obtained in this investigation for esters, sulfides, acetals, amines, and lactone demonstrates the point. Some of these compounds have been reported previously (17, 22, 26), but quantitative data are vague. In any case, it appears that the volatile carbonyls are a contributing factor to the over-all flavor picture, and additional studies involving a large number of cheeses will be required before a definite relationship can be established between individual components of the carbonyl mixture and Cheddar flavor.

The occurrence of a series of odd-numbered methyl ketones suggests the presence of mold growth in the cheese. In no instance was mold growth evident, yet the ketones were found in every cheese studied. It was concluded, therefore, that these compounds are normal products of the curing phenomenon.

The identification of methional in these studies lends support to the previous suggestion (15) that it may be an important flavor compound in Cheddar cheese. It is significant that Jackson (13) found that the retention time of methional is the same as cheese-like aroma when Cheddar volatiles are separated by gas chromatography. Jackson (13) also supports the report (15) that proper concentrations of methional yield a cheese-like aroma. Mechanisms for the conversion of methionine to methional and of other amino acids and keto acids to some of the identified aldehydes have been discussed elsewhere (15, 18).

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COCONUT-LIKE FLAVOR DEFECT OF MILK FAT. IV. DEMONSTRATION OF δ-DODECALACTONE IN THE STEAM DISTILLATE FROM MILK FAT[1]

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SUMMARY

Liquid-partition chromatography, using various solvent systems on paper and in packed columns together, with gas-liquid chromatography, enabled the identification of δ-dodecalactone in the steam distillate from fresh milk fat. It appears that this lactone occurs in the distillate at a lower level than does δ-decalactone. Because of its characteristic fruity flavor and odor properties, δ-dodecalactone is considered to be of possible significance in flavor changes of processed and stored dairy products. A useful column partition system for chromatographic separation of certain aliphatic lactones was revealed in the course of the investigation.

Previous papers (3, 4) in this series have been concerned with the presence, origin, and flavor significance of δ-decalactone in certain dairy products. This paper deals with δ-dodecalactone, a compound that is structurally analogous to the C_{10} lactone, except that it contains 12 carbons. Flavor change in heated and stored dairy products involves the formation of minute quantities of many different flavor compounds. These lactones appear to be a significant aspect of this phenomenon.

EXPERIMENTAL PROCEDURE

The means of preparing fresh anhydrous milk fat and of steam-distilling the fat to generate and recover flavor compounds have been described (4, 5). In general, 1 to 2 liters of milk fat was distilled at 150 to 200° C., pressures of 0.25 to 5 mm. Hg, and for 2 to 3 hr. Steam equivalent to 100 to 200 ml. of water was passed through the fat in each distillation and was condensed together with the volatile organic matter that it contained in two ethanol–dry ice traps. On completion of a distillation, the dry ice traps were allowed to thaw and the distillate was recovered. Invariably, all but 1 or 2 ml. of the distillate was recovered in the first trap.

The neutral organic material in the steam distillate was isolated as follows: The initial slight acidity of the distillate was neutralized to the phenolphthalein end point with aqueous 10% Na_2CO_3 solution. After overlaying the neutralized distillate with ethyl ether that had been used to rinse any residual matter from the dry ice traps, the aqueous phase was saturated with salt and extracted three to five times with ethyl ether. The combined extracts were evaporated under reduced pressure to yield 1 to 2 ml. of a clear, oily liquid with a light

Received for publication October 30, 1959.

[1] Authorized for publication as Paper No. 2412 on October 26, 1959, in the Journal Series of the Pennsylvania Agricultural Experiment Station.

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yellow color and a pronounced odor of heated milk fat. This material was consistent in yield and the indicated properties from distillation of one lot of fat to the next. It was prepared in repeated operations as needed for the following experiments involving identification of δ-dodecalactone.

Paper chromatography. Preparation and paper chromatography of lactone hydroxamic acid derivatives from the distillate extract were carried out by the methods of Keeney (2, 3). To differentiate more clearly between esters and lactones present, there being some glyceride material in the distillates, the partially neutralized hydroxylamine reagent (2) was used since it reacts preferentially with lactones. The hydroxamates were paper-chromatographed using Keeney solvent systems No. 1 and 3, and the spots were revealed by spraying with a 1:1 aqueous mixture of 5% HCl and 5% $FeCl_3$. The distillate extract was also paper-chromatographed as such, using the Heulin solvent system for 2,4-dinitrophenylhydrazones (1). After developing and drying, the chromatograms were lightly sprayed with hydroxylamine reagent (unneutralized) and 15 min. later with an aqueous 3:1 by volume mixture of 10% HCl and 10% $FeCl_3$. Representative R_f values for authentic δ-dodecalactone and an unknown corresponding to it from the distillate extract are given in Table 1 for the three different chromatographic conditions. For comparative purposes, data on δ-decalactone also are given. Spots corresponding to this lactone invariably were present and although no special effort was made to quantitate results, such spots were consistently larger and more intense than those for the C_{12} lactone.

Column partition chromatography. During the course of an earlier investigation dealing with the methyl ketones in the distillate from milk fat (5), it was noted that some of the fractions from the partition column used to separate dinitrophenylhydrazones carried strong odors suggestive of lactones. This evidence that the particular column could be used to fractionate lactones was explored further. The column in question employs celite as suport, nitromethane as the immobile phase, and hexane saturated with nitromethane as the mobile phase. Using columns based on 30 g. of celite, and the paper chromatographic methods previously described to detect components in the column eluates, retention volumes for a number of authentic lactones and esters were determined. These data are presented in Table 2.

TABLE 1

R_f values from paper chromatograms by several solvent systems for the hydroxamates of an unknown and two authentic lactones

Solvent system	Unknown	δ-Dodecalactone	δ-Decalactone
Keeney No. 1[a]	0.43	0.43	0.15
Keeney No. 3[b]	0.90	0.91	0.84
Huelin[c]	0.72	0.72	0.58

[a] Equilibrating solvent—aqueous phase from 5:5:1 by volume mixture of benzene, water, and glacial acetic acid. Developing solvent—10% solution of glacial acetic acid in benzene. R_f values are decidedly influenced by the presence of water in the developing solvent.

[b] Equilibrating solvent—aqueous phase from 5:3:5:1 by volume mixture of benzene, isopropanol, water, and glacial acetic acid. Developing solvent—upper phase from the mixture.

[c] Equilibrating solvent—methanol phase from 2:1 by volume mixture of n-heptane and methanol. Developing solvent—upper phase from this mixture. In using this solvent system, the lactones were chromatographed as such and after development were converted to hydroxamates.

TABLE 2

Threshold volumes of compounds separated by liquid partition column chromatography[a]

Compound	Volume
	(ml.)
Trihexanoin	80
Methyl butyrate	100
δ-Dodecalactone	175
δ-Decalactone	275
γ-Decalactone	300
δ-Nonalactone	450
Tripropionin	550
γ-Valerolactone	750

[a] Employing hexane saturated with nitromethane as mobile phase and 30 g. of celite coated with 33 ml. of nitromethane as stationary phase.

To confirm further the presence of δ-dodecalactone, the neutralized distillate extract from 1 liter of milk fat was fractionated on the partition column. The fraction corresponding in retention volume to authentic δ-dodecalactone was recovered and evaporated under reduced pressure. The odor of the residue appeared to be identical to that of authentic δ-dodecalactone. Paper chromatography of this residue, both as such and after reaction with hydroxylamine reagent, revealed the presence of a component behaving in the manner of the C_{12} lactone.

Gas chromatography. The gas chromatographic analyses were made on a Barber-Coleman Model 10 instrument. Initial evaluations with authentic δ-lactones revealed that polyester-coated column packing (LAC-2-R 446, a diethylene glycol adipate resin) is unsuitable for the purpose. The lactones appear to undergo transesterification with the resin and even large undiluted samples (2 μl.) could not be detected at high sensitivity settings of the instrument. Satisfactory results were obtained when Apiezon L was used as the stationary phase. Figure 1 shows the correspondence in retention time of authentic δ-dodecalactone and the unknown from the milk fat distillate after purification by column partition chromatography. As a matter of interest, the position of δ-decalactone is shown in the reference analysis. The operating conditions for these analyses were as follows: 8 ft. glass column, 6-mm. inside diameter, packed with 100-140 mesh celite coated with 20 wt. % Apiezon L.; carrier gas, argon at a pressure of 30 p.s.i. and an approximate flow rate of 150 ml/min; column maintained at 220° C., detector at 265° C., and sample heater at 300° C. The reference sample injected into the machine was 1 μl. of a 0.1% solution of δ-dodecalactone in hexane. The concentration of the unknown was not determined.

DISCUSSION

δ-Dodecalactone has fruity, peach-like odor and flavor qualities. At times, deteriorated dry whole milk may have such a flavor. In a few instances, according to observers at this laboratory, this type of off-flavor in the product seems to predominate over the characteristic coconut-like flavor defect. Such practical importance as δ-dodecalactone may have in stored dairy products probably involves this phenomenon. Although δ-decalactone yielded more intense and larger spots on paper chromatograms than did δ-dodecalactone when these lactones were recovered from milk fat distillates, precise quantitative data were not secured.

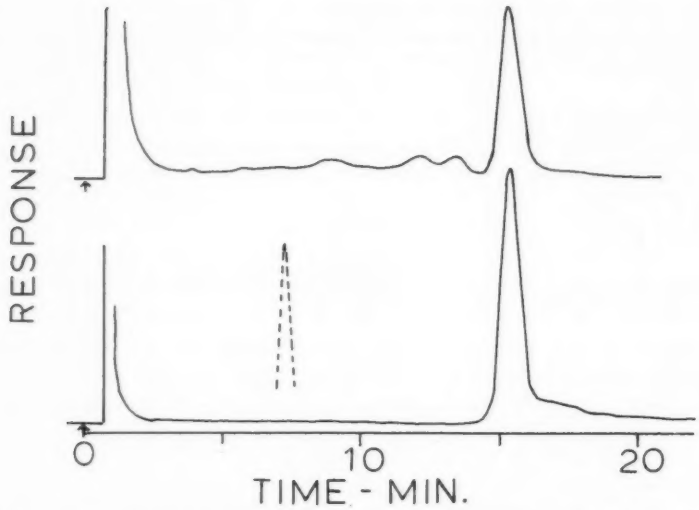

FIG. 1. Gas chromatograms of a purified unknown component from the steam distillate of milk fat (upper), and of δ-dodecalactone (C_{12}, lower). The position of δ-decalactone (C_{10}) under these conditions also is shown.

Moreover, the potential to produce one or the other of these lactones may vary among milk fat samples. A further consideration in the matter is that information on the relative flavor potency of the two lactones is lacking.

Evidence of several other lactones in the distillate from milk fat was secured during the study. Two of these apparently occur at much lower concentrations and move more slowly on paper chromatograms than either the C_{10} or C_{12} lactone.

A quantitative method for measuring the lactones in question in milk and milk products is badly needed. Such a method would facilitate basic studies of origin and flavor significance of such compounds and might solve the more practical problem of screening milk supplies for potential to produce lactones as a result of processing and storage. A factor that complicates development of such a method is the relative ease with which the lactones form in milk fat on heating or storage. Although previous investigations (3, 4) have indicated that these lactones are undetectable in strictly fresh, unheated fat, the possibility of their formation during the manipulative work in preparing samples of sufficient size for analysis is a distinct problem. It appears that gas chromatography involving use of a highly sensitive instrument may afford a solution to this problem. In the present investigation, 1 μl. of 0.1% δ-dodecalactone in hexane did not approach an ultimate challenge to the sensitivity of the chromatography unit. It is this order of sensitivity that will be needed to enable quantitative analysis for trace amounts of volatile compounds in biological systems.

ACKNOWLEDGMENTS

The authentic lactones for this research were supplied through the kindness of Fermenich and Co., New York, and Unilever Ltd., Port Sunlight, England.

The paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces and has been assigned No. 1059 in the series of papers approved for publication. The views or conclusions contained in the report are those of the authors, and are not to be construed as necessarily reflecting the views or endorsement of the Department of Defense.

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EFFECTS OF LACTIC CULTURES ON ACIDITY AND FIRMNESS OF COTTAGE CHEESE COAGULUM[1]

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SUMMARY

Nonfat dry milk reconstituted to give 11% solids-not-fat was made into Cottage Cheese coagulum at 90° F., by adding 7% starter and 1 ml. rennet/1,000 lb. of skimmilk. Seventeen different cultures were used.

A close relation was found between pH of curd and curd strength produced by active cultures. Regardless of their activity, the cultures showed essentially the same relation between curd strength and decrease in pH after coagulation.

The 17 cultures varied widely in their relations between curd strength and titratable acidity of whey, and in their relations between pH of curd and titratable acidity of whey.

High titratable acidity produced by some cultures at the A-C end point was associated with floating curd during heating. The phenomenon seems to be explained by varied production by the different cultures of weakly ionized acids such as carbonic and acetic acid.

The data explain why occasional vats of milk do not coagulate at normal titratable acidity values, and indicate that it is not always desirable to use the same culture for making both Cottage Cheese and Cultured Buttermilk.

Heinemann (8) has shown that different lactic cultures affect the relationship between titratable acidity of whey and the strength of Cottage Cheese curd. He suggested that such differences in curd firmness reflected varied proteolytic activity of the lactic cultures.

This paper is a report of further investigations of the effect of different cultures on the relationships between curd strength, titratable acidity of whey, and pH of Cottage Cheese coagulum.

EXPERIMENTAL METHODS

Analysis of covariance (12) was used to compare the effect of different cultures on the acidity and firmness of curd at various stages after coagulation.

Seventeen commercial lactic cultures were used. Each culture was tested on two different days of its effect on curd strength. Four of these cultures, C, Z, R, and H, were transfers from the cultures of Heinemann (8) numbered E1, F1, F11, and F12, respectively. All cultures were tested within a period of four days.

During the study it was found that at least one culture had changed markedly in its characteristics between the first and second trials. This change was ap-

Received for publication November 25, 1959.

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parent in rate of acid development, in relations between pH of curd to titratable acidity of whey, pH of curd to curd strength, and titratable acidity of whey to curd strength. For this reason, in the statistical analysis, each of the two trials with the 17 cultures was considered a separate culture, making a total of 34 cultures.

A single lot of low-heat commercial nonfat dry milk (NDM) was used as the source of skimmilk in these experiments. This NDM was phosphatase- and coliform-negative; it had a standard plate count of 100,000/g after incubation for 48 hr. at 32° C. (1). The NDM was reconstituted at the rate of 11.0 lb. of powder to 89.0 lb. of cold tap water and stored overnight at 40° F. before using.

For testing each culture, 1,200 ml. of skimmilk was warmed in a beaker to 90° F. and mixed with 85 ml. of the culture under test. The mixture was held in a 90° F. water bath for 30 min., after which rennet was added at the rate of 1 ml/1,000 lb. of skimmilk. This skimmilk-culture-rennet mixture was divided equally among six 250-ml. beakers which were covered and replaced in the water bath at 90° F.

One beaker was watched carefully for the first signs of coagulation. When coagulation had been detected, the time was noted and this beaker was removed from the bath; measurements of curd strength, pH of the curd, and titratable acidity of the whey were made, in that order, as quickly as possible. At intervals thereafter, the same observations were made on the five other beakers, so that measurements were obtained in each series over fairly wide ranges of pH, titratable acidity of whey, and curd strength.

For each trial, the pH of coagulation at zero curd strength was estimated by using the pooled regression coefficient of these two factors. The pooled regression coefficient of curd strength on pH of coagulation for the 34 trials was −197.14 g. curd strength per pH unit. The titratable acidity of whey at coagulation was estimated by extrapolating to the estimated pH of coagulation the curves of the relationship between titratable acidity of whey and pH of curd. The time of coagulation was estimated by extrapolating to the estimated pH of coagulation the curves showing relationship between pH of curd and time.

Development of visible bubbles of gas in curd from certain starters reduced curd strength; data on such samples were discarded.

Curd strength measurements were made with the Cherry-Burrell curd tension meter and knife (2).

Measurements of pH were made at 90° F., using a Beckman Model G pH meter with external glass and reference electrodes.

Titratable acidity measurements of whey were made according to the recommendations of Doan *et al.* (5), using a 17.6-ml. Babcock milk pipette.

RESULTS

Figure 1 shows the relationship between curd strength and pH of the curd, whereas Figure 2 shows how curd strength is related to titratable acidity of whey. Each of these graphs is based upon 194 observations of the 34 cultures. For Figures 1 and 2, the correlation coefficients are −.85 and .59, respectively.

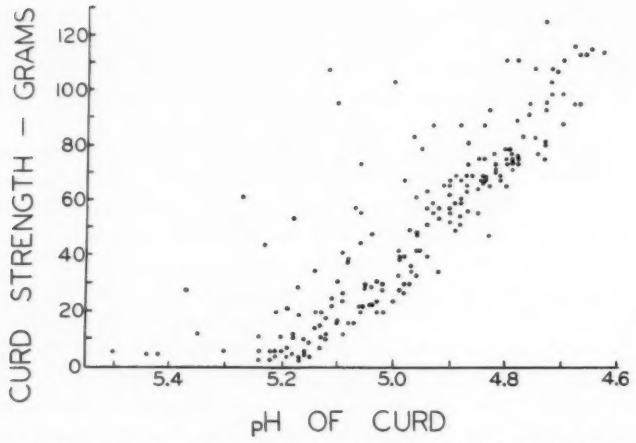

FIG. 1. Relationship between curd strength and pH of curd developed by 17 cultures in two series of trials; the number of observations totaled 194 and approximated five or six per culture per trial.

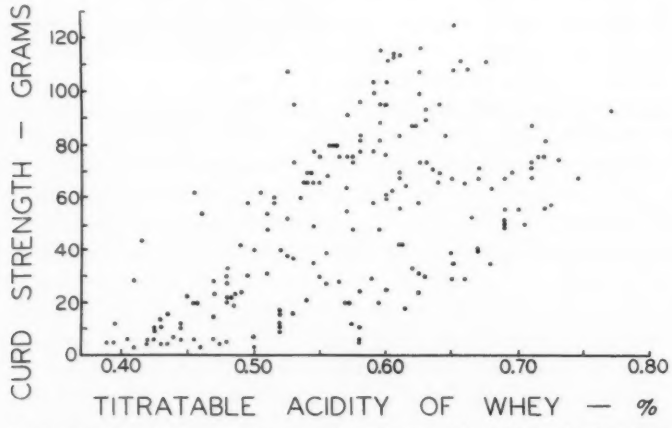

FIG. 2. Relationship between curd strength and titratable acidity of whey from curd developed by 17 cultures in two series of trials; the number of observations totaled 194 and approximated five or six per culture per trial.

At the same titratable acidity curd strength varied widely with the different cultures. Some showed curd strength values of over 100 g. at titratable acidities of 0.55% in the whey; others did not even coagulate the skimmilk until the titratable acidity of the whey had reached, or exceeded, 0.55%. These data confirm Heinemann's observations (8).

Table 1 presents the analysis of covariance (13) for the effect of the 34 cultures on the relationship of curd strength to pH of curd. The analysis shows

no significant effect of different cultures on the relation between curd strength and the decrease in pH after coagulation. The data plotted in Figure 3 indicate for all cultures a marked dependence of curd strength on the decrease in pH after coagulation; this is expressed by the correlation coefficient of 0.97. The rate of change in pH in these pH ranges varied fourfold between the most active and least active cultures. Therefore, time elapsed after coagulation would not appear to be a determining factor in the relationship between curd strength and decreases in pH.

TABLE 1

Analysis of covariance for effect of different lactic cultures on the relation between curd strength *(Y)* and pH of curd *(X)* during development of acidity after coagulation

Source of variation	Degrees of freedom	Mean square
Deviations about individual regressions	126	66.9
Differences among regression coefficients	33	101.1 n.s.[a]
Deviations about pooled regression	159	74.0
Differences among adjusted means	33	1,324.4**[b]

[a] n.s.—There were no significant differences ($F = 1.51$) among the 34 regression coefficients of curd strength on pH. A pooled regression coefficient of −197.14 g. per pH unit was obtained.

[b] **—There were highly significant differences (1% level, $F = 17.9$) among the 34 means of curd strength adjusted for pH values at coagulation.

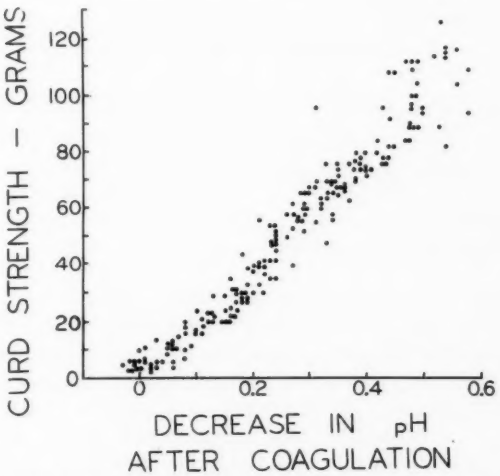

FIG. 3. Relationship between curd strength and the decrease in pH after coagulation in 34 trials with 17 different cultures.

Although the cultures did not affect the relationship between curd strength and decrease in pH after coagulation, the analysis of covariance in Table 1 shows that the cultures differed in curd strength when adjusted for the pH of coagulation. A possible explanation for this effect is suggested by Figure 4. Here the estimated pH values of coagulation, which varied from 5.12 to 5.53 in the 34 trials, are plotted against the time elapsing between the addition of

rennet and the time of coagulation. This relationship suggests that those cultures which change the pH of skimmilk slowly allow more time for rennet action, so that the skimmilk coagulates at a higher pH; the same result would be obtained if more rennet were used with a more active culture.

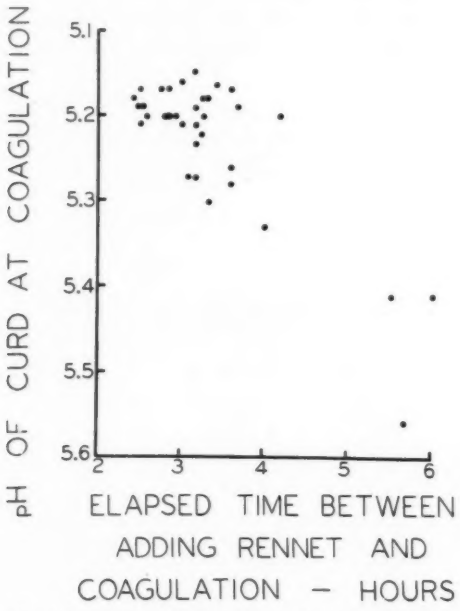

FIG. 4. Relationship between the pH of the curd at coagulation and the elapsed time between the addition of rennet and coagulation.

When only those cultures showing satisfactory activity were considered, their effect on the relationship between curd strength and the pH of curd was small. The arbitrarily selected standard of activity used was attainment of pH 5.0 at 90° F. within 5 hr. after adding the starter culture. Figure 5 shows the close relation ($r = 0.97$) between 129 observations of curd strength and pH of the curd for the 23 out of 34 cultures which showed satisfactory activity. However, analysis of covariance in Table 2 shows that the difference in the curd strength of the 23 cultures adjusted for pH of coagulation was significant at the 5% level but not at the 1% level. The range of the estimated pH values at coagulation for the 23 trials was only 0.07 pH unit.

The analysis of covariance indicated highly significant differences of curd strength on titratable acidity of whey ($F = 6.40$). Since the regression coefficients differed, it was not possible to test differences among cultures for curd strength adjusted for differences in titratable acidity, using a common regression. The titratable acidity of whey from different cultures at coagulation varied from 0.38 to 0.58%.

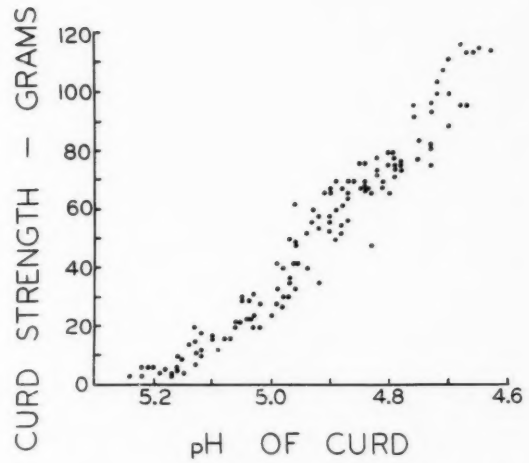

FIG. 5. Relationship between curd strength and pH of curd for 23 cultures which developed pH 5.00 in 5 hr. or less at 90° F. after addition of culture.

Figure 6 shows the relationship between curd strength and tritratable acidity of whey for the same 23 active cultures used in Figure 5. A correlation coefficient of .52 was obtained. Analysis of covariance indicated highly significant differences ($F = 5.62$) among the regression coefficients of curd strength on titratable acidity of whey after coagulation of the 23 active cultures used in Figure 5. At coagulation, the estimated values of titratable acidity of whey varied from 0.41 to 0.58%.

Figure 7 shows for all 34 cultures the relationship ($r = 0.83$) between curd strength and the increase in titratable acidity of whey after coagulation. Duplicate trials of Cultures A and G are plotted in Figure 7. Regression coefficients for duplicate trials, using Cultures A and G, were 516 and 602 g. for A, and 177 and 251 g. for Culture G, per 1.0% increase in titratable acidity. These are extreme differences which show that the effect of the culture on this relationship is significant.

TABLE 2

Analysis of covariance for effect of lactic cultures in 23 trials[a] on the relation of curd strength *(Y)* and pH of curd *(X)*

Source of variation	Degrees of freedom	Mean squares
Deviations about individual regression	83	50.5
Differences among regression coefficients	22	51.7 n.s.[b]
Deviations about pooled regression	105	50.8
Differences among adjusted means	22	96.0*[c]

[a] The active cultures used in these trials developed pH 5.00 in 5 hr. or less after adding the starter culture.

[b] n.s.—$F = 1.02$, not significant.

[c] *—$F = 1.89$, significant at 5% level.

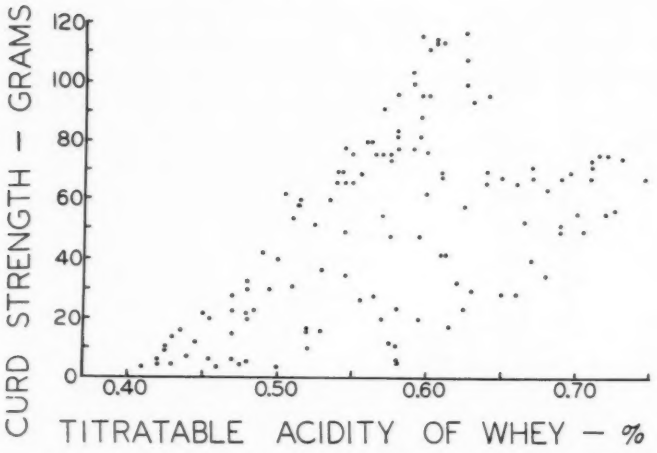

FIG. 6. Relationship between curd strength and titratable acidity of whey for 23 trials with active cultures.

Differences in significance of the effect of various cultures on the relationships shown in Figures 3 and 7 are explained in Figure 8, where values for titratable acidity of whey are plotted against measurements of pH of curd. If the curves of Figure 8 were parallel, different cultures would be expected to show the same relationship between curd strength and increase in titratable acidity of the whey after coagulation. Culture differences revealed in Figure 8

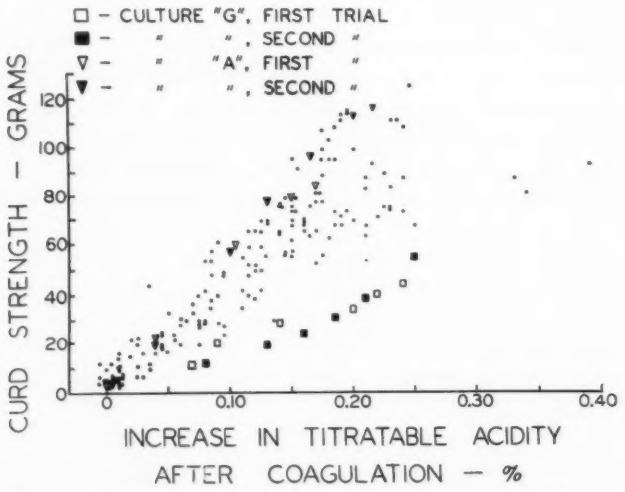

FIG. 7. Relationship between curd strength and increase in tritratable acidity of whey after coagulation in 34 trials with 17 different cultures.

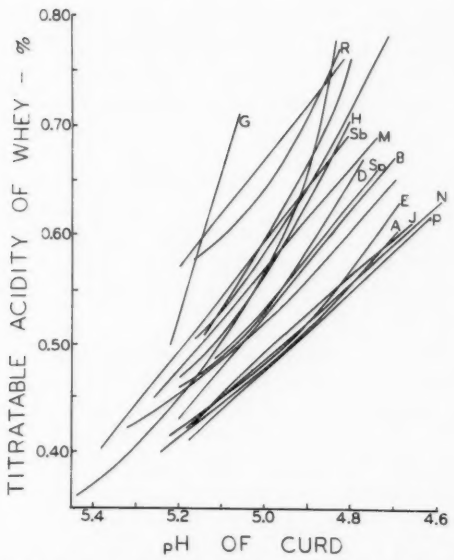

FIG. 8. Relationship between titratable acidity of whey and pH of curd for 17 cultures. All curves except those for Cultures Sa and Sb are the average of two trials.

are similar to differences in pH vs. titratable acidity curves shown by Czulak and Hammond (4) for *Streptococcus diacetilactis, Streptococcus lactis,* and *Streptococcus durans.* Czulak (3) had attributed differences in the titratable acidity–pH relationships of *S. lactis* and *S. diacetilactis* to production of carbon dioxide by the latter organism.

Differences in the relationship between pH of the curd and titratable acidity of whey for the commercial lactic cultures were very wide. At a pH of 5.1 in curd, for instance, the titratable acidity of whey varied from 0.44% for Culture P to 0.66% for Culture G. At pH 4.75, the titratable acidity varied from 0.57% for Culture P to 0.76% for Culture W; extrapolation of the curves for other cultures, such as R and T, indicated even higher values of titratable acidity at pH 4.75. At a titratable acidity of 0.55% in the whey, the pH varied from 5.25 for Culture C to 4.79 for Culture P.

Data in Table 3 show that cultures which developed high titratable activities of whey at the relatively constant pH of the A-C end point also produced curd which floated during heating. Curd which sank during heating had titratable acidities of whey ranging from 0.57 to 0.64%; but curd which tended to float during heating showed titratable acidities of 0.67% or more in the whey.

The method used to determine the floating tendency of Cottage Cheese curd during cooking was similar to that described by Sandine *et al.* (11), except that the curd was cut vertically with a spatula into strips approximately ½ by ½ in. in cross section.

TABLE 3

Titratable acidities of whey at the A-C end point for different cultures and the tendencies to floating curd at end of curd heating operation

Action of curd	No. of cultures	No. of trials	Range of acidity of whey at A-C end point
			(%)
Sinking curd	8	19	0.57–0.64
Floating curd	5	12	0.67–0.79

It was assumed that the production by some cultures of weakly ionized acids, such as carbonic, could account for differences in acidity and floating tendencies listed in Table 3, and for the varied relationship between pH and titratable acid of cultures shown in Figure 8.

Most activity tests for cultures measure the rate of change of titratable acidity during initial acid development. Cultures for Cottage Cheese manufacture must maintain satisfactory activity over a much wider range of pH. In the Horrall-Elliker (9) activity test, Cultures G, J, and T showed the following activities: 0.48, 0.45, and 0.52%. The times required to reach the A-C end point at pH 4.75–4.78 in pasteurized skimmilk were 7.3, 4.4, and 4.8 hr., respectively. Factors such as the heat treatments of the skimmilk and temperatures of incubation may have been involved, but differences in activity over different pH ranges and differences in pH–titratable acidity relationships contributed to the differences in culture activity noted above. Observations indicate the desirability of using the A-C test as an activity test for cultures for Cottage Cheese; this application has been described and recommended by Hales (7).

DISCUSSION

Observations of lower titratable acidity of one culture, as compared to that of another when both have the same pH value, can be explained by the probability that the second produced a greater proportion of weakly dissociated acids, such as carbonic or acetic acid. Carbonic acid is largely undissociated at pH 5.0 and below. Thus, the production of this weakly ionized acid by a culture would not lower the pH appreciably and yet would contribute significantly to the titratable acidity test. Sandine, Anderson, and Elliker (10) observed that their Culture 30 produced 1,027 microliters of gas from 3 ml. of skimmilk when incubated for 4 hr. at 30° C. If it is assumed that all of this gas were carbon dioxide, then Culture 30 should have had a titration value of 0.12% (expressed as lactic acid) higher than another culture at the same pH that had developed only lactic acid.

The relation between culture activity and the pH of coagulation, shown in Figure 4, indicates a marked effect of pH on the evidence of rennet action. If pH were not important, all cultures would have coagulated the skimmilk at the same time and the line of best fit would have been parallel to the Y-axis. Whether the effect of pH is on the enzymatic activity of the rennin or on the susceptibility of the enzymatically altered casein to coagulation, or both, is still an open question.

It is also of some interest that cultures showing widely varying activity did not affect the relation after coagulation between pH of the curd and the curd

strength. It is apparent that pH is much more important than time in the development of strength in Cottage Cheese curd.

The results of these experiments with active cultures show better correlation between pH and curd strength than between titratable acidity and curd strength. This suggests that variations in curd strength at any given titratable acidity can be largely accounted for by differences in hydrogen ion concentration. The possibility of differences in proteolytic actions of the different cultures associated with these results can not be forgotten, as Heinemann suggested (8). The work of Van der Zant and Nelson (13) indicated that starters would be expected to produce negligible amounts of soluble nitrogen in 12 hr. of incubation and only small, though measurable, amounts of tyrosine in as short a time as 4 hr. What effects, if any, such proteolysis might have on strength of curd formed in approximately 5 hr. by the action of rennet and acid is uncertain, but it is probably of a minor nature, considering the experimental evidence of this study.

Some of the observations and relationships considered in this study have special practical significance.

When different, active, mixed strain commercial cultures were considered, a close relation was established between curd strength and pH of curd, and a very poor relation between curd strength and titratable acidity of whey. Measurement of pH appears to be a more reliable index of the handling and cooking properties of Cottage Cheese curd than titratable acidity of whey.

The composition of milk has long been known to affect the relationship between pH and titratable acidity. The observations of this study have shown that this relationship is affected by cultures to such an extent that it has a significant effect in Cottage Cheese manufacture. If a gas-forming culture were used, normal titratable acidity standards for cutting would be too low to attain the correct pH for cutting, as indicated by the A-C end point. Cutting at high pH values could cause the matting in curd produced by gas-forming cultures, the phenomenon observed by Sandine *et al.* (11). The obvious solution to this problem, as proposed by Sandine and his coworkers, would be to select cultures which do not produce sufficient gas to cause floating curd or the formation of gas bubbles in curd during heating of the curd.

Observations of titratable acidity of whey from curd at the A-C end point might serve as a presumptive test for the ability of a starter to produce weakly ionizing acids, such as acetic and carbonic. A homofermentative, single strain culture should be the standard for comparison with the starter to be tested. The starter able to produce the weakly ionizing acids and carbon dioxide would show a higher titratable acidity in the whey at the A-C end point than the single strain culture.

This relationship between pH at coagulation and titratable acidity should also be of interest to those who use the same culture for both buttermilk and Cottage Cheese. Starters which produce high aroma in buttermilk may be unacceptable for Cottage Cheese because they may also produce large amounts of carbon dioxide. These experiments with different cultures suggest that pH determinations can play an important part in controlling the quality of buttermilk.

Viscosity, wheying-off, and sourness of taste may be more closely related to pH than to titratable acidity. For different cultures, like those shown in Figure 8, wide variations in the pH values of different buttermilks would be expected at the same titratable acidity.

Finally, the results of this study may also explain the occasional vats of skimmilk for Cottage Cheese that do not coagulate, even though the titratable acidity is identical to or higher than that normally found in milk at coagulation. The extreme insolubility of casein around pH 4.7 to 4.6 makes it very unlikely that coagulation or some degree of curd formation would not occur at that pH level.

ACKNOWLEDGMENT

We are grateful to Burdet Heinemann, Producers' Creamery Company, Springfield, Missouri, for supplying several of the cultures used in this study and for his review of the manuscript.

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ACCURACY OF A LACTOMETRIC FIELD TEST FOR MEASURING SOLIDS-NOT-FAT CONTENT OF MILK[1, 2]

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SUMMARY

The solids-not-fat (SNF) content of milk can be measured with sufficient accuracy in the field by DHIA supervisors using the small Watson-pattern lactometer if readings are made at 102 ± 0.5° F. In the first of two trials, comparisons between field and laboratory measurements showed small differences due to improper temperature control. No practical differences between field and laboratory were observed in the second trial. The mean difference between lactometer readings in the field and in the laboratory was 0.28 lactometer degree. Accuracy to 0.5 lactometer degree is comparable to the accuracy of the Babcock test for fat. Correlations between field and laboratory tests, between duplicate samples in the field, and between duplicate samples in the laboratory were .96, .98, and .98, respectively, for the first trial, and .97, .99, and .99, respectively, for the second trial.

Simple, accurate, and relatively inexpensive methods of testing for the per cent of SNF in milk have been developed for routine use in the laboratory. Adapting these methods to field conditions provides the potential for testing large numbers of cows in much the same manner as the Babcock test is used in the DHIA program.

This study measures the accuracy of the small Watson-pattern lactometer under field conditions as compared with its use in the laboratory.

EXPERIMENTAL PROCEDURE

Equipment. The lactometers used were the small type reported in 1957 by Watson (4) and later shown by Madden and Brunner (3) to agree closely with the gravimetric method for determining the solids content of milk. Portable water bath kits were used to maintain the milk at the required temperature of 102° F. These kits were insulated and contained a thermostatically controlled heating unit, a pump for circulating the water, and removable racks which held 36 samples of milk in glass cylinders.

Testing procedure. Five lactometer kits (lactometers, water baths, and 4-oz. sample bottles) were placed with six DHIA supervisors in five Michigan counties. One kit was used alternately by two supervisors in the same county. Four of these supervisors conducted the test at home; the other two conducted the test from farm to farm.

The DHIA supervisor obtained a 4-oz. sample of milk from each cow, which provided enough milk to conduct the Babcock test before performing the lactometer test. The milk was heated to approximately 102–105° F. by immersing

Received for publication October 15, 1959.

[1] Journal Article No. 2510 from the Michigan Agricultural Experiment Station.

[2] These studies were supported in part by the Dairy Cattle Research Branch of the Agricultural Research Service, USDA, and the Holstein-Friesian Association of America.

the sample bottle of milk in water at about 115–120° F. The milk was transferred to glass cylinders and placed into the preheated water bath. Lactometer readings were made at 102° F. Although the lactometers were graduated in 0.5 lactometer degree, readings were estimated and recorded to the nearest 0.1 lactometer degree.

Method of comparison. The DHIA supervisors were given a training session of one-half day to acquaint them with the equipment and the lactometric procedure. Additional assistance was provided the first time each supervisor conducted the test in the field. Quadruplicate samples of milk were obtained from every sixth cow until a total of six cows had been sampled. Duplicate samples were analyzed in the field by the DHIA supervisor and the other two samples were analyzed by a technician the next day under laboratory conditions. Preservatives were not used. The DHIA supervisor usually tested the duplicate sample in a separate group following the testing of the original samples. Duplicates were not identified to the technician and were tested in random order. The same procedure was repeated 2 mo. later, in Trial II, with the exception that the samples were obtained from different herds. All of the herds sampled were registered Holsteins. Because of a difference in the number of cows tested for SNF by each supervisor, experience prior to the second trial ranged from 88 to 1,200 samples per supervisor.

RESULTS AND DISCUSSION

Trial I. In every comparison in Trial I the lactometer reading in the laboratory was higher, by an almost constant amount, than that observed in the field. Temperature readings were not recorded in this trial, but were suspected of varying from 102° F. by more than the recommended ± 0.5° F. In some cases, failure to temper the lactometer led to lowering the temperature of the milk by as much as 2° F.

Analysis of variance showed differences significant at the .01 probability level between each of the supervisors and the laboratory technician. Partitioning of the variance into its components gave the results presented in Table 1. Differences between samples accounted for over 90% of the variance for three of the six supervisors. The within-tester differences accounted for 4% or less of the vari-

TABLE 1

Components of variance for field-laboratory comparison of lactometers—Trial 1

		DHIA Supervisors											
		1		2		3		4		5		6	
Source	D.F.	Var.	%	Var.	%	Var.	%	Var.	%	Var.	%	Var.	%
Sample	5	3.24	96	1.60	91	.11	19	.85	66	.88	68	2.28	96
Location	1	.09	3	.12	7	.42	75	.42	32	.36	28	.08	3
S × L	5	.00	0	−.01[a]	0	.01	2	.01	1	−.02[a]	0	.00	0
Within-tester	12	.03	1	.04	2	.02	4	.01	1	.05	4	.02	1
Total	23	3.36		1.76		.56		1.29		1.29		2.38	

[a] Negative components were considered zero for computing totals and percentages.

ance for all testers, whereas differences due to location (field or laboratory) accounted for less than 7% of the variation for three supervisors. Temperature differences between the laboratory and the field are suspected of being the major source of error causing the high variance due to location for the other three supervisors.

Correlations between field and laboratory, between duplicate samples in the field, and between duplicate samples in the laboratory were .96, .98, and .98, respectively.

Trial II. The temperature of the milk was recorded to the nearest 0.1° F. immediately following all lactometer readings recorded in Trial II, since temperature was suspected of being involved in the differences noted in Trial I. The temperature ranged from 101.2 to 102.8° F. None of the testers allowed the temperature to vary by more than 0.9° F.; some testers held the temperature variation to 0.3° F. Ninety-two and six-tenths per cent of the samples analyzed were within the range of 102 ± .5° F.

Analysis of variance showed differences significant at the .01 probability level between three of the supervisors and the laboratory and significant at the .05 probability level between two supervisors and the laboratory. Adjusting for temperature as suggested by Watson (4) resulted in reducing the differences so that they were significant at the .01 probability level for only one supervisor and significant at the .05 probability level for two supervisors.

In an analysis of variance, significance depends on both the size of the difference and upon the number of observations involved. In this experiment, the number of observations was sufficient to make a difference of 0.2 lactometer degree appear significant. The need for this much accuracy in the lactometer reading is questionable. Since the per cent of fat has a marked effect on the specific gravity of milk, it is used with the lactometer reading in calculating the per cent of total solids and SNF. It is known that Babcock tests for fat may differ by 0.1% or more in comparative tests by different technicians (1, 2). An error of 0.1% of fat results in a difference of 0.13% in the calculated total solids. An equivalent error of 0.5 lactometer degree results in a difference of 0.13% in the calculated total solids. Since this degre of precision is accepted for the Babcock test for fat, there is little justification for greater accuracy in the lactometer test for total solids or SNF. In Trial II only 8% of the samples differed by more than 0.5 lactometer degree between field and laboratory measurements. The mean difference was 0.28 lactometer degree.

The variance components for Trial II (Table 2) indicate no practical difference between field and laboratory data. Variance attributable to differences between samples accounted for 83 to 97% of the total variance, whereas 0–6% of the variance was due to location. The within-tester variation ranged from 0–11%, with no apparent reason for the increased within-tester error for one supervisor. However, it should be pointed out that differences between duplicate readings made by the laboratory technician are included in this component and contribute approximately one-half of the error. Correcting the data for temperature (Table 3) reduced the variance due to location in three cases and within testers

TABLE 2
Components of variance for field-laboratory comparison of lactometers—Trial II

		DHIA Supervisors											
		1		2		3		4		5		6	
Source	D.F.	Var.	%	Var.	%	Var.	%	Var.	%	Var.	%	Var.	%
Sample	5	.73	92	1.72	97	1.27	97	2.46	93	.30	83	2.78	96
Location	1	.04	5	.04	2	.01	1	.17	6	−.01[a]	0	.09	3
S × L	5	−.01[a]	0	.01	.5	−.01[a]	0	.02	1	.02	6	.01	0
Within-tester	12	.02	3	.01	.5	.02	2	.01	0	.04	11	.02	1
Total	23	.79		1.80		1.30		2.66		.36		2.90	

[a] Negative components were considered zero for computing totals and percentages.

TABLE 3
Components of variance for field-laboratory comparison of lactometers—Trial II corrected for temperature

		DHIA Supervisors											
		1		2		3		4		5		6	
Source	D.F.	Var.	%	Var.	%	Var.	%	Var.	%	Var.	%	Var.	%
Sample	5	.73	94	1.79	98	1.20	99	2.45	93	.30	83	2.81	99
Location	1	.02	3	.02	1	.01	.5	.16	6	−.01[a]	0	.01	0
S × L	5	.00	0	.01	.5	.00	0	.02	1	.02	6	.01	0
Within-tester	12	.02	3	.01	.5	.01	.5	.00	0	.04	11	.02	1
Total	23	.77		1.83		1.22		2.63		.36		2.85	

[a] Negative components were considered zero for computing totals and percentages.

in one case. The reductions were only slight and from a practical standpoint temperature corrections are not needed as long as the lactometer readings are made within ± .5° F. of 102° F.

In both trials, over 90% of the differences between duplicates, including both field and laboratory, were not more than 0.4 lactometer degree. The largest difference between duplicates in either trial was a 0.6 lactometer degree difference for one set of duplicates. The differences between field and laboratory measurements ranged between 0.2 and 1.2 lactometer degrees in Trial I and between 0 and 0.9 lactometer degree in Trial II. Only three of the differences between the field and the laboratory in Trial II were more than 0.5 lactometer degree. The frequency distribution of differences for Trial II are presented in Table 4.

Correlations between field and laboratory, between duplicate samples in the field, and between duplicate samples in the laboratory were .97, .99, and .99, respectively. The magnitude of the correlation between field and laboratory data in both trials indicates that for all practical purposes the small Watson-pattern lactometer is as accurate under field conditions as it is in the laboratory. The high correlations between duplicate samples, both in the laboratory and in the field, indicate that duplicate lactometric determinations add little information and, therefore, are not necessary to estimate accurately the SNF content of milk.

TABLE 4
Cumulative distribution of differences between duplicate lactometer readings—Trial II

	DHIA Supervisors		Laboratory		Field-laboratory	
Differences[a]	No.	%	No.	%	No.	%
0	6	16	9	24	3	8
.1	19	51	23	62	10	26
.2	28	75	33	89	15	40
.3	32	86	36	97	23	61
.4	35	94	37	100	29	77
.5	36	97			34	91
.6	37	100			34	91
.7					35	94
.8					36	97
.9					37	100

[a] Differences measured in lactometer degrees.

CONCLUSIONS

Temperature control is necessary in conducting the lactometer test. The recommendation of reading the lactometer in milk held at 102 ± 0.5° F. must be strictly adhered to if the lactometer is to be used accurately. Correcting for temperature within this range adds little or nothing to the accuracy of the test.

The small Watson-pattern lactometer is accurate in the field when used with a water bath kit equipped for maintaining the temperature at 102 ± 0.5° F.

Duplicate lactometric determinations are not necessary to accurately estimate the SNF content of milk.

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BLOOD AND RUMEN CHANGES FOLLOWING THE INTRA-RUMINAL ADMINISTRATION OF GLYCOGENIC MATERIALS [1]

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SUMMARY

An over-all study was made of blood and rumen changes taking place in fistulated steers following intraruminal administration of propylene glycol, sodium propionate, sodium lactate, calcium lactate, mixed lactates, and sucrose. Additional specific studies were made on the relative proportions of administered sodium lactate that were fermented and absorbed, and on the glycogenic effect of various propionate-butyrate mixtures.

Propylene glycol gave the greatest response in blood glucose. Propionate increased blood glucose more than any of the lactate treatments. Sucrose did not escape fermentation and rumen intermediates were not favorable for increasing blood glucose.

Lactate absorption increased while lactate fermentation decreased with increasing solubility of the lactate administered. Lactate fermentation resulted in the greatest increase, based on molar ratios, in propionic acid followed by butyric acid and lastly by acetic acid. Higher rumen concentration of lactate increased the percentage loss by absorption and decreased the percentage loss by fermentation. The loss by fermentation remained nearly constant when rumen concentrations ranged from 200 to 1,000 mg.%, with little absorption occurring below 200 mg.%

Intraruminal dripping of propionate-butyrate mixtures showed gradual increases of blood glucose and decreases of blood ketones as the proportion of propionate increased.

A number of glycogenic materials which can be fed have been suggested for the control of ketosis in dairy cows. Some of these, such as lactates and propionates, are normally produced in the rumen. Other compounds suggested, such as propylene glycol, are related chemically. The sugars themselves have also received some consideration. More information is needed on how these materials are metabolized by the ruminant, in order to evaluate them as glycogenic agents for ruminants.

The metabolism of lactic acid by the ruminant has been studied by a number of workers. There is general agreement that lactic acid is produced in the rumen shortly after feeding (1, 9, 24, 32). There is evidence to suggest that at least a considerable proportion of the lactic acid produced in the rumen is fermented to other acids, particularly propionic, but also some butyric and acetic (9, 10, 13, 24, 32). Some difference of opinion exists regarding absorption of lactate. Some workers (23) have observed little or no absorption. Others have observed increases in blood lactic acid after administering high levels of lactate (13). The effectiveness of lactates in the control of ketosis has been studied to a limited extent (28, 29, 30).

Propionic acid injected or administered orally to ruminants has been shown to be strongly glycogenic (7, 14, 16, 27). Propionates have been used for the

Received for publication December 4, 1959.

[1] The data published in this paper are from a thesis presented by the senior author to the graduate school, Cornell University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, September, 1957.

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treatment (11, 31) and prevention (26) of ketosis. Propylene glycol was suggested as a treatment for ketosis by Johnson (15) and has been used successfully for this purpose (20, 22). Oral doses of glucose have given little or no increase in venous blood glucose (4, 8, 12, 24).

EXPERIMENTAL PROCEDURE

The study was divided into three separate trials. The first involved the administration of a number of glycogenic materials, at various levels, to fistulated steers. Changes taking place in the blood and rumen were measured. The second trial involved a study of the absorption of sodium lactate, using a technique involving simultaneous in vivo and in vitro fermentation. The third trial involved a study of the ketogenic properties of various propionate-butyrate mixtures, since these acids were produced in greater quantities than acetic acid following lactate administration, and have shown indications of stronger glycogenic and ketogenic properties.

During Trial 1, glycogenic materials were administrated to four fistulated Holstein steers weighing about 800 lb. These animals were maintained on a diet of mixed hay plus 3 lb. of grain per day in two feedings. Intervals of 1 wk. were allowed between successive treatments. The treatments were given in the morning following the last feeding by about 15 hr. No feed or water was allowed during the sampling period. In addition to water as a control, the materials used were sodium propionate, sodium lactate, calcium lactate, a mixture of sodium and calcium lactate, propylene glycol, and sucrose. Each of these materials was given at 0.5-, 1-, and 1.5-lb. levels. These 19 treatments were administered once, into the fistula of each steer.

Adjustments for the concentration of the sodium lactate syrup were made so that actually 0.5, 1, and 1.5 lb. of sodium lactate were used. Adjustments were not made for the pentahydrate form of calcium lactate. Thus, when using calcium lactate the actual dosage of lactate ion was only 73% as great as when using sodium lactate; when using the mixture, only 87% as great as when using the sodium lactate.

Rumen samples were taken from the ventral portion of the rumen and blood samples were taken from the jugular vein just before administration. These were designated as the zero-hour samples. Additional rumen samples were taken at 0.5, 1, 2, 3, 4, 6, 8, and 10 hr. Additional blood samples were taken at 1, 2, 4, 6, and 8 hr.

To investigate absorption in Trial 2, the equivalent of 1.5 lb. of sodium lactate was administered once to each of the same animals used in Trial 1. Steers I and III received this amount about 19 hr. after the last feeding. Steers II and IV received this amount about 4 hr. after the last feeding. Natural ruminal mixing was allowed for 1 hr. Duplicate samples were taken hourly thereafter. After being subsampled for analysis, these samples were incubated in a 39° C. water bath and gassed with carbon dioxide. Thus, fermentation was allowed to continue, but no loss of lactate by absorption was taking place. Additional subsamples were taken at 0.5 and 1 hr. from the incubating sample, after which

the sample was discarded. In all, three subsamples were taken from each of the duplicate samples removed from the rumen. This cycle of sampling was repeated each hour until lactate disappeared from the rumen.

In Trial 3, propionate-butyrate mixtures were administered to three goats on a hay and concentrate ration. A period of four days was allowed between each administration. Water and solutions of sodium propionate, sodium butyrate, and mixtures of these two salts were given as indicated in Figure 1. Each of

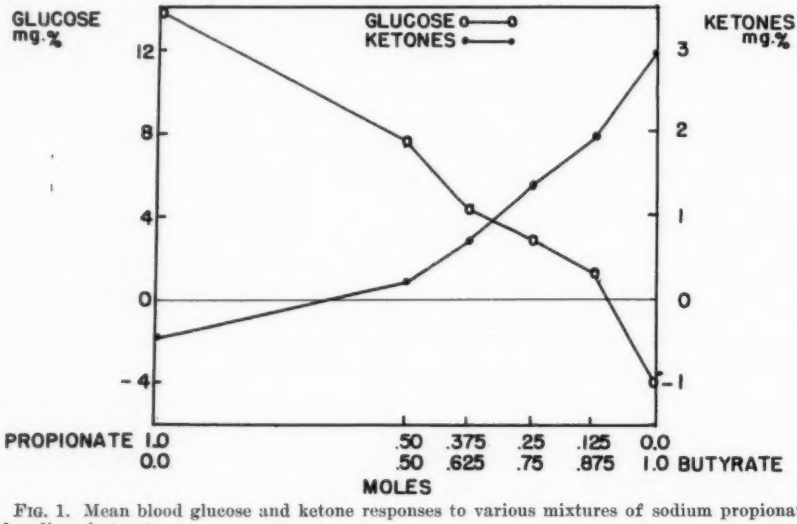

FIG. 1. Mean blood glucose and ketone responses to various mixtures of sodium propionate and sodium butyrate.

these was mixed in one liter of water and dripped into the rumen over a period of 2 hr. Each goat received each treatment once. The blood sample taken before the intraruminal drip started was called zero-hour. Additional samples were taken from the jugular vein every hour until 8 hr. after the start of administration.

All rumen samples were filtered through one layer of gauze. Thirty milliliters were mixed with 0.6 ml. of saturated mercuric chloride solution. This was stored under refrigeration until analysis. Blood samples of sufficient size were taken to allow 10 ml. for pipetting. Proteins were precipitated by tungstic acid on the same day and the filtrates were stored under toluene with refrigeration.

Blood glucose was run on the filtrate by the method of Benedict (5). Lactic acid was analyzed by the method of Barker and Summerson (2). Blood ketones were converted and distilled as acetone by the method of Behre and Benedict (3), and the acetone was determined by the method of Block and Bolling (6). The fatty acids were separated by the direct chromatographic procedure of Keeney (17).

RESULTS

Blood changes. The statistical analyses of blood glucose changes and blood lactic acid changes following dosage with the various compounds are given in

TABLE 1
Analysis of variance on changes in blood constituents following intraruminal dosage with glycogenic materials

Source	d.f.	Mean square	
		Glucose	Lactic Acid
Animal	3	632	459
Treatment	18	644[a]	874[a]
Starvation vs. others	1	189	11
Compound	5	1,212[a]	2,165[a]
Level	2	391	1,618[a]
Compound × level	10	455	166
Error	54	276	192

[a] Represents significance ($P<.01$).

Table 1. The hourly values were corrected for the zero-hour concentration on the individual animal for that sampling sequence. These corrected values were coded by adding 20 for glucose and adding 10 for lactic acid. The coded values were summed over the remaining five sampling times. These sums were used in the analysis. The unweighted mean changes are given in Table 2. Each value in

TABLE 2
Mean changes in blood constituents following intraruminal dosage with glycogenic materials

Compound given	Glucose	Lactic Acid
	(mg. per 100 ml.)	
Propylene glycol	6.0[a]	.....
Sodium propionate	3.7[ab]	−0.2[d]
Sodium lactate	3.4[abc]	0.7[d]
Mixed lactate	1.7[bc]	1.0[d]
Calcium lactate	1.2[bc]	−1.0[d]
Sucrose	0.6[c]	−0.7[d]

Values followed by same superscript are not significantly different ($P>.05$), based on Duncan's test.

Table 2 represents an average increase above the concentration at zero-hour for five samples at each of three levels on each of four steers, or a total of 60 determinations. They are unweighted because they represent five blood samples taken at unequal intervals over 8 hr. This procedure averages any rapid peak effect. It does not show maximum increases, but does give an indication of the ability of the material to raise blood sugar for periods of 8 hr. The blood ketones were determined on only one randomly selected animal of the four. In no case did the blood ketone level rise up to 4 mg.%. No statistical analysis was made as a result of these small differences which fall in the normal range.

Rumen changes. The statistical analyses on the rumen acids, lactic, propionate, butyric, and acetic, are presented in Table 3. In addition to the hourly volatile acid values being corrected for the zero-hour concentration on the individual animal for that sampling sequence, a second correction was made on the basis of a series of control samples taken at the same times when water was given. This second correction allowed for dilution during administration and decline due to starvation. The values after these two corrections are assumed to repre-

TABLE 3
Analysis of variance on changes in rumen acids following intraruminal dosage with glycogenic materials

Source	Lactic Acid		Fatty acids			
				Mean square		
	d.f.	Mean square	d.f.	Propionic	Butyric	Acetic
		(thousands)		*(thousands)*	*(thousands)*	*(thousands)*
Animal	3	1,129	3	238 [a]	10	196
Treatment	15	10,458 [b]	17	4,300 [b]	44 [b]	159 [a]
Starvation vs. others	1	6,067 [b]				
Compound	4	19,967 [b]	5	10,815 [b]	106 [b]	215 [b]
Level	2	16,967 [b]	2	2,905 [b]	72 [b]	31
Compound × level	8	4,626 [b]	10	1,321 [b]	7	157
Error	45	646	51	84	13	83

[a] Represents significance ($P<.05$).
[b] Represents significance ($P<.01$).

sent the contribution of the treatments to the acid concentrations in the rumen. These corrected values were summed over the remaining eight sampling times. These sums were then coded by adding 700. The analysis was run on these coded values. There are differences in degrees of freedom for lactic acid compared to volatile acids, because volatile acid concentrations were corrected for decline in concentration due to starvation, and lactate response following propylene glycol was not included because of interference in the colorimetric analysis. The unweighted average increases of rumen acids are presented in Table 4. Each value

TABLE 4
Mean increases in rumen acids following intraruminal dosage with glycogenic materials

Compound given	Rumen acids			
	Lactic	Propionic	Butyric	Acetic
	(mg. per 100 ml.)			
Sodium propionate	0 [d]	348 [e]	19 [k]	1 [n]
Propylene glycol	.....	76 [f]	25 [jk]	22 [lmn]
Calcium lactate	143 [c]	66 [f]	50 [h]	36 [lm]
Mixed lactate	227 [b]	65 [f]	43 [hi]	19 [lmn]
Sodium lactate	397 [a]	59 [f]	41 [hi]	11 [mn]
Sucrose	29 [d]	31 [g]	34 [ij]	47 [l]

Values followed by same superscript are not significantly different ($P>.05$), based on Duncan's test.

in Table 4 represents an average increase above the concentration at zero-hour for eight samples at each of three levels on each of four steers, or a total of 96 determinations. For lactic acid these means represent concentrations directly, because no corrections were made for the insignificant amounts present in the rumen before treatment. They are unweighted because they represent eight rumen samples taken at unequal intervals over 10 hr.

Absorption of lactate. The samples taken from the in vitro fermentations in Trial 2 did not indicate that the rate of fermentation changed during the 1-hr. period, so the rate of fermentation was assumed to be constant for the hour in

vitro fermentation period. The loss by fermentation represented the decrease in concentration during the 1-hr. fermentation in the artificial rumen. Loss by absorption represented the difference between the concentrations in the artificial rumen and the rumen at the end of the hour. These relations ignore the effects of dilution by saliva, passage of lactic acid, or changes in rumen volume. They assume that the rate of fermentation in the artificial rumen is identical to that in the rumen. On this basis the results are presented in Table 5. Considerable

TABLE 5

Hourly disappearance of lactate from the rumen after administration of 1½ lb. sodium lactate based on concentration at the start of each hour

Concentration range in rumen	No. of observations	Time after administration	Loss by fermentation		Loss by absorption	
			% of total hourly loss	Mean mg. % per hour	% of total hourly loss	Mean mg. % per hour
(mg. per 100 ml.)		*(hr.)*				
Above 1,200	2	0–1	4	37	96	923
1,000 to 1,119	2	0–1	40	192	60	289
800 to 999	2	0–1	49	124	51	131
600 to 799	4	1–2	53	132	47	116
400 to 599	10	1–3	69	123	31	56
200 to 399	6	2–4	77	130	23	39
0 to 199	8	3–5	93	98	7	7

variation occurred in the individual observations. One time in the 34 observations the concentration found after 1 hr. of fermentation in vitro was greater than the initial concentration. Three times the rate of fermentation in vitro exceeded the loss from the rumen.

Ketogenic properties of propionate-butyrate mixtures. The hourly glucose values were corrected for the zero-hour concentration on the individual animal for that sampling sequence. The corrected values were coded by adding 20. The coded values were summed over the remaining eight samples. These sums were used in the analysis. Although the three animals showed significant ($P<.05$) differences in both elevation and slope, the single regression is $Y = 14.6 - 16.6X$ where Y = mean mg.% blood sugar response for 8 hr. after the start of dosage and X = moles sodium butyrate given with $1 - X =$ moles sodium propionate given. The regression coefficient is very highly significant ($P<.001$), with a standard deviation of $\pm$ 2.4. The hourly ketone values were corrected for the zero-hour concentration on the individual animal for that sampling sequence. These corrected values were coded by adding 3 and multiplying by 10. The coded values were summed over the remaining eight samples. These sums were used in the analysis. Since differences in either elevation or slope due to animals were not significant ($P>.05$), the pooled regression is $Y = 3.4X - 1$ where Y = mean mg.% blood ketone increase for 8 hr. after the start of dosage and X = moles sodium butyrate given with $1 - X =$ moles sodium propionate given. The regression is very highly significant ($P<.001$), with a standard deviation of $\pm$ .76. In neither case were the curvilinear components significant. The data are pre-

sented graphically in Figure 1, where each point represents the average change from concentration at zero-hour for eight samples on each of three goats, or a total of 24 determinations.

DISCUSSION

Either administered lactate or lactic acid accumulating from rumen fermentation could be absorbed from the rumen or converted to other acids. Conversion of lactic acid to other acids could change its glycogenic effect. Administered lactate remained in the rumen for an average of 3 hr. following dosage with 0.5 lb. and 6 hr. following either 1 or 1.5 lb. The nine individual curves of rumen lactate disappearance rate plotted on the basis of three sources at three levels did not differ significantly ($P>.05$). The three curves for sources which were pooled over levels did not differ significantly ($P>.05$). Neither did the three curves for levels pooled over sources differ significantly ($P>.05$). In other words, the source or the level of lactate made no difference in the rate of disappearance when rate was expressed as per cent of lactic acid present. On the basis of increases in blood glucose and rumen lactic acid, the order of rank from greatest to least response was sodium, mixed and calcium salts. Using Duncan's test, the only differences that were significant ($P<.05$) were those that occurred in rumen lactic acid. This significance no longer existed when the response was adjusted by covariance for differences in the amount of lactate ion given. On the basis of increase in rumen propionic, butyric, and acetic acids, the order of rank from the greatest to least was calcium, mixed and sodium salts. On this basis the rank was the inverse of that above. This may lend support to the finding of Hueter *et al.* (13), that more of the sodium lactate may be absorbed directly and that more of the calcium salt may be converted to rumen acids.

In the lactate absorption trial there were large variations in the concentration in the rumens of animals of equal size. All animals were given equal doses of sodium lactate and were on the same ration. Close agreement of the duplicate samples taken from the rumen would indicate that adequate mixing had occurred during the 1-hr. equilibration time. The per cent of the total decrease attributable to absorption decreased as the rumen concentrations decreased. Likewise, the mean drop in concentration per hour resulting from absorption decreased as rumen concentrations became lower. On the contrary, the average drop in concentration per hour due to fermentation remained nearly constant when the initial concentration in the rumen varied from 200 to 1,000 mg.%. Low average decrease due to fermentation occurred at rumen concentrations below 200 mg.% and above 1,200 mg.%, which occurred shortly after administration. The per cent of the total decrease attributable to fermentation increased as the rumen concentration decreased.

If the decreases in concentration due to absorption are individually summed on each animal, the percentage of lactate absorbed varies from 22 to 78% when 1.5 lb. sodium lactate was given all at once. More absorption occurred on the animals showing the higher rumen concentrations. This technique may be biased, in that lactate-utilizing bacteria may be increasing as rumen concentration de-

creases. However, the apparent decrease in concentration due to fermentation was nearly constant for a considerable range in rumen concentrations, and the decrease in concentration due to fermentation was smaller in the animals dosed 4 hr. after feeding than it was in those dosed 19 hr. after feeding. Lactate utilizers should have been present in larger numbers at 4 hr. after feeding. Lactic acid seldom reaches a peak concentration in the rumen above 200 mg.% and usually is present for only 4 hr. after feeding. On this basis the absorption of lactate would make only a small contribution to the total energy on most natural rations. Since the amounts of fatty acids formed from lactate seemed to be influenced by the solubility, lactic acid formed in the rumen may react in a manner different from sodium lactate.

Considerable portions of therapeutic or prophylactic doses of lactate are fermented to other acids. The lactate fermentations produced ruminal fatty acid increases in the following molar ratios: propionic, 0.86; butyric, 0.51; and acetic, 0.37. Assuming two moles of lactate are needed to form one mole of butyrate, the value for butyrate would have to be doubled to show the relative amounts of lactate used in the formation of these acids. Based on the trial using molar ratios of propionate and butyrate, the ratio formed from lactate fermentation would result in a slight increase in both blood glucose and ketones. Any lactate absorbed would increase the glycogenic effect of that portion fermented.

Increasing the butyrate in the molar ratio reduced the blood glucose concentration and increased the blood ketone concentration. Only butyrate alone depressed blood glucose and only propionate depressed blood ketones. Contrary to these results, Kleiber *et al.* (18) and McCarthy (21) have demonstrated that the activity of labeled butyrate can be converted into carbohydrate. This apparent contradiction may be resolved according to the conclusion of Weinman *et al.* (33), that no net synthesis of carbohydrate could occur from even-numbered carbon fragments. They postulate that radioactivity gains entrance into carbohydrates by an influx scheme which involves an exchange of Kreb's cycle intermediates. Assuming no acidosis occurred in this trial, these results are in conflict with the results of Kronfeld *et al.* (19), that intraruminal infusion of sodium butyrate, which gives no increase in acidosis, resulted in an increase in blood glucose.

The blood glucose increase following propionate was greater than that following lactate treatments. The caloric content of the propionate is greater than lactate. Probably a more important reason is that propionate is more nearly an end product of rumen fermentation than lactic acid. The lactate was partly absorbed and partly fermented. That part if its fermentation end products having an even number of carbon atoms would be primarily ketogenic. Although absorption of lactate did occur, blood lactic acid values showed little increase when lactate was placed in the rumen.

Of all the materials administered, propylene glycol caused the greatest increase in blood sugar. The reason for propylene glycol having a greater effect than sodium propionate could be due to the fact that a greater molar quantity was given because of the smaller molecular weight of the glycol. It is also possible

that absorbed propylene glycol may shift the equilibrium in some Embden-Myerhoff reactions to favor glucose synthesis rather than utilization. The authors have observed in using propylene glycol for treatment of ketosis that there is a rather rapid increase in blood glucose, but that the response in milk production does not parallel this increase in blood glucose as it does in the case of treatment with propionate. Rumen fatty acid values indicate considerable production of propionic acid in the rumen. This indicates that blood glucose increases are probably a result of propylene glycol absorbed directly, plus the absorption of fermentation products.

At the levels of sucrose given it would be expected that no sucrose would escape fermentation. Lactic acid accumulated in the rumen for periods up to 4 hr. after administration, indicating that part of the fermentation proceeded through a stage of lactic acid. The level of lactate accumulating in the rumen was so low that most of the lactate was probably converted to other acids. Since more acetic acid was produced from sucrose than from any of the lactate treatments, it would indicate that much of the sucrose was not fermented through lactic acid. Sucrose for the treatment of ketosis could serve as an energy source, but did not result in rumen intermediates which had the favorable glycogenic-ketogenic ratio required of a good ketosis treatment.

ACKNOWLEDGMENT

The authors are indebted to Dr. John J. Jonas, Research and Development Division, National Dairy Products Corporation, for supplying the calcium lactate used in these studies.

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THE OXIDATION-REDUCTION POTENTIAL OF RUMEN CONTENTS[1,2]

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SUMMARY

The relationship between the E_h and pH of rumen fluid was found to be represented by the equation $E_h = E_o - 0.06$ pH. Investigation of the reductive capacity of rumen fluid removed from cows on various rations indicated:

1. A very small proportion of the reduced substances found in rumen fluid is associated with the bacterial cells.
2. The amount of reducing substances is significantly higher several hours after feeding than at other times during the day.
3. There is a definite relationship between the ration fed and the reducing capacity of rumen fluid removed at different times during a 24-hr. period. This relationship is in agreement with the fact that concentrates are digested more quickly than roughages.

On the basis of the above observations, it is proposed that the reductive characteristics of rumen fluid can be used as an index of fermentation rate in much the same manner as pH is presently used.

Perhaps the greatest difficulty in dealing with biological systems generally, and particularly with the bacterial populations found in the reticulo-rumen of cattle, arises from the fact that we do not have rigidly defined static systems possessing fixed characteristics. Early investigations by many workers in the field of bacteriology pointed out that aerobes are capable of metabolizing normally when the redox potential of their environment lies within the range from about +0.4 v. to −0.2 v. Anaerobes require a potential between +0.05 v. and −0.4 v. for normal metabolism. The ability to metabolize within these ranges is a manifestation of the characteristics of the enzyme systems that the various organisms possess. That is, aerobes are capable of metabolizing in highly oxidative media because a high proportion of their enzymes are capable of working efficiently while the potential is high. Anaerobes, on the other hand, depend largely upon enzymes which do not operate normally until the media are partially reduced (7). Since the redox potential has such an important effect upon the enzymatic processes essential for normal metabolism by bacteria, it seems important that the reductive characteristics of the rumen environment be known.

Very little information is available concerning the redox potential of rumen contents. The potential of the in vitro system of Marston (10) was in the vicinity of −380 mv. at pH 7, with rapid fluctuations of ± 30 mv. The potential of rumen contents treated with various levels of aureomycin and penicillin ranged from −193 to −288 mv. (8). Broberg (1, 4) found that the potential of rumen contents taken from animals on hay and grain diets did not differ significantly from

Received for publication November 10, 1959.

[1] Published with the approval of the Director of the Michigan Agricultural Experiment Station as Journal Article No. 2530.

[2] The data reported here were taken from the thesis submitted by R. L. Baldwin to the Graduate School, Michigan State University, in partial fulfillment of the requirements for the Master of Science degree.

that observed in samples from animals on pasture. The E_h of samples removed at various times after feeding was fairly constant at −140 mv. The potential was quite variable in a number of bloat cases (2). In view of the wide variation in potential observed in these cases of bloat, Broberg (2) suggested that enzymatic processes other than normal govern the course of events in some cases of bloat. Large amounts of oxygen bubbled through rumen contents failed to cause more than a temporary rise in potential in vivo. No correlation was noted between CO_2 production and oxygen consumption in vitro (3).

The purpose of this study was to characterize the reductive substances of the rumen contents and to determine the effect of added oxidizing and reducing compounds on the rumen fermentation.

EXPERIMENTAL PROCEDURE

Three cows, T-3, T-25, and K-139, fitted with the screw-cap plastic fistula plugs described by Hentschl *et al.* (6), were used in this investigation. T-3 and K-139, respectively, were maintained on hay rations of 20 lb. of timothy daily and 20 lb. of first-cutting alfalfa daily. T-25 was fed 10 lb. of first-cutting alfalfa hay and 10 lb. of a 15.8% crude protein grain mixture daily. The experimental rations were fed daily at 7 A.M. for at least 3 wk. before the investigation was initiated. The animals had free access to water at all times.

To facilitate the determinations and to investigate the nature of the reducing substances in rumen fluid, the samples were separated into several fractions by centrifugation. The large feed particles (Fraction C) were separated from strained rumen fluid (Fraction A) by centrifugation at 200 G for 3 min. The resulting supernatant fluid (Fraction B) was then subjected to a force of 27,600 G for 15 min. The supernatant from this treatment was designated as Fraction D and the residual, Fraction E.

A Beckman automatic titrimator fitted with saturated calomel, glass and platinum electrodes was used to measure the electromotive force (EMF) and the pH. Ten per cent sulfuric acid was used to adjust the pH and as the titrant for the determination of the relationship between E_h and pH. Acid bichromate and acidic ceric sulphate were used as titrants. All samples were kept under a nitrogen atmosphere during the titrations. Oen droy of 0.002% methylene blue was added to each sample to act as a mediator during the measurement of E_h.

The second part of this experiment was initiated to investigate other factors that contribute to the stability of the rumen environment. The method chosen for this purpose was the addition of various oxidative and reductive compounds to rumen fluid, incubated in vitro for short periods of time, and the measurement of the effects these compounds had upon the fermentation. The additives employed were oxygen, methylene blue, DPPD (diphenyl-para-phenylene diamine), ascorbic acid, cysteine, and thioglycollate.

Two hundred and fifty milliliters of whole rumen fluid collected at 10:30 A.M. were used for each trial. The gas produced was collected and its composition determined. Oxygen and carbon dioxide were measured by absorption and methane by combustion. The volatile fatty acids were determined by the method

of Keeney (9). Ammonia was determined colorimetrically by the permutite method described by Hawk *et al.* (5).

RESULTS AND DISCUSSION

The data presented in Table 1 indicate that a linear relationship exists between the E_h and pH of rumen contents. The regression coefficient, b, calculated by the method of least squares, is 0.0595 v. and the standard error (deviation about the regression line) calculated by the method of Snedecor (11) was ±00.0061 v. The relationship between the pH and E_h of rumen fluid can be expressed by the relation $E_h = E_o - 0.06$ pH, where E_h is the potential in volts at

TABLE 1
Relationship between E_h and pH

1		2		3		4		5		6	
pH	EMF[a]	pH	EMF	pH	EMF	pH	EMF	pH	EMF	pH	EMF
6.2	−260	6.8	−300	6.6	−280	7.2	−380	7.0	−300	7.2	−270
5.0	−200	6.0	−250	5.3	−200	5.9	−300	5.8	−250	6.3	−200
4.3	−150	5.1	−200	4.5	−150	5.1	−250	5.1	−200	5.2	−150
3.4	−100	4.2	−150	3.7	−100	4.2	−200	4.2	−150	5.0	−100
2.4	− 40	3.6	−100	2.6	− 50	3.5	−150	3.4	−100	4.0	− 50
2.2	+ 30	2.6	− 50	1.8	0	2.5	−100	2.4	− 50	2.8	0
		1.9	0			1.8	− 50	1.5	0		
						1.0	0				

[a] EMF expressed in millivolts potential between calomel electrode and rumen fluid.

any pH; E_o is the potentia' at pH 7, and 0.06 is the regression coefficient. These results are not in agreement with those presented by Broberg (3), who found no consistent relationship between E_h and pH when he acidified rumen contents either by stages or abruptly. A possible explanation for the difference is that Broberg worked with a living population, whereas dead rumen bacteria were used in this experiment. Broberg (3) reported that when the pH was within the physiological range, 10 to 15 min. passed before the system came to equilibrium. When the organisms are dead and methylene blue is used as a mediator, equilibrium is reached very quickly. These facts indicate that the effect of pH upon E_h is modified by living organisms. Examination of Broberg's data indicates that the potential observed in his system immediately after acidification is the same as the potential that would be predicted by the above equation.

The E_h of water extracts of alfalfa hay and cornmeal was +0.250 v. at pH 7. To eliminate the possibility that the results of titrations would be influenced by the amount of water-soluble feed constituents present, the end point for the titrations reported in Tables 2 and 3 was chosen as +0.200 v. at pH 7. The data reported in Table 2 show that approximately 8.9% of the total reductive capacity of rumen fluid is associated with the bacterial cells (Fraction E), whereas 47.2 and 37.5%, respectively, are associated with the large feed particles (Fraction C) and supernatant colloidal material (Fraction D). It is concluded, on this evidence that, under the anaerobic conditions that exist in the rumen, the bacteria utilize their substrate as an electron acceptor.

The E_h and the amounts of reducing compounds found in Fraction B at three different times of the day in cows on three different rations are reported in

TABLE 2

Proportion of reducing substances present in the various fractions of rumen fluid at 10:30 A.M.

Fraction		A Whole	B Super	C Feed	D Super	E Cells
Average milliliters per fraction		50	36	14	34	2
Milliequivalents reducing substance per fraction	1	1.95	0.90	1.00	0.70	0.15
	2	2.20	1.10	1.00	0.90	0.20
	3	2.40	1.10	1.20	0.80	0.15
	4	2.00	1.05	0.90	0.80	0.25
Mean		2.14	1.04	1.03	0.80	0.19
% of total		100	47.6	47.2	37.5	8.9

TABLE 3

Potentimetric titrations of Fractions B

Cows	Rations	6 A.M.	10:30 A.M.	6 P.M.
T-25	Hay and grain	1.55[a]	2.65	1.64
T-3	Timothy hay	1.25	2.33	1.75
K-139	Alfalfa hay	1.78	2.21	2.01

[a] Mean of four values expressed as milliequivalents reducing substance per 50 ml. Fraction B.

TABLE 4

E_h and pH of Fraction A

Time		6 A.M.			10:30 A.M.			6 P.M.		
		E_h[a]	pH	E_h[b]	E_h[a]	pH	E_h[b]	E_h[a]	pH	E_h[b]
Cow	T13	−125	7.3	−107	−155	6.4	−191	−75	7.0	−75
		−65	7.1	−59	−145	6.3	−187	−125	7.3	−107
	T-25	−125	7.2	−113	−135	6.4	−171	−95	7.2	−83
		−135	7.1	−129	−155	6.7	−173	−15	7.1	−9
	K-139	−105	7.4	−81	−165	6.8	−177	−95	6.2	−143
		−35	7.2	−23	−85	6.2	−133	−155	7.4	−131

[a] Uncorrected E_h expressed in millivolts.
[b] E_h corrected to pH 7.0, expressed in millivolts.

Tables 3 and 4. The method for analysis of variance in multiple classifications presented by Snedecor (11) was applied to these data. Ration, time, and the ration-by-time interaction were designated as the sources of variance. The amount of reducing substances present at 10:30 A.M. was significantly higher than the amounts found at 6 A.M. and 6 P.M. The effect of time of day upon the uncorrected E_h values approached significance at the 0.05 confidence level. When the E_h values were corrected to pH 7, according to the formula presented earlier, the effect of time of day upon the E_h was significant at the 0.01 level of confidence. The E_h was always lowest at 10:30 A.M.

The type of ration fed did not generally influence the E_h or the reductive capacity. However, the interaction between ration and time was a significant (P 0.01) source of variance, due to the relatively high amounts of reducing substances present in samples from T-25 at 10:30 A.M. and the continual presence of moderate amounts of reducing substance in the hay-fed cows. This observation

correlates very well with the accepted fact that concentrates are digested more quickly than roughages.

The identities of the compounds which heretofore have been referred to as reducing substances are mostly unknown. The linear characteristics of titration curves also determined on the samples indicate that the reductive capacity of the rumen fluid is not due to the presence of any one compound but many compounds with different normal redox potentials.

The amounts of reducing substances in rumen contents are not very high, at least not high enough to be totally responsible for the stable conditions that exist there. For example, it would take only five equivalents of a strong oxidizing agent to raise the potential of 175 lb. of rumen contents to +0.200 v., if the reducing capacities measured in Table 1 were the only stabilizing factor in rumen fluid. A potential of +0.200 v. is inconsistent with normal anaerobic metabolism.

Oxygen was the only additive which measurably affected the fermentation. The results for the trials where oxygen was employed are summarized in Table 5.

TABLE 5

Effect of methylene blue and oxygen upon in vitro fermentation

	Control		MB		MB + O_2		O_2	
	1	2	1	2	1	2	1	2
E_h at zero time *(mV.)*	−65	−55	−65	−55	−65	−55	−65	−55
E_h at 3 hr. *(mV.)*	−85	−105	−55	−75	−85	−55	−55	−105
Total gas production (ml.)	225.6	303	224.1	316.6	215.2	309.5	223.6	336.9
CO_2 *(%)*	68.0	71.1	67.9	73.0	79.0	74.8	77.0	73.6
O_2 utilized *(%)*	.....	.....	.....	.....	36.0	28.0	31.8	30.5

No effect upon volatile fatty acid or ammonia production was noted and these values have not been reported in Table 5. Although the only significant difference due to the action of oxygen was the increase in carbon dioxide attributable to the oxygen uptake, there are some interesting points about these data. The fact that oxygen did not raise the potential or cause changes in the end products indicates that the microbial population in the rumen is capable of maintaining a constant environment, even in the presence of a strong oxidizing agent such as oxygen. This observation lends support to the hypothesis that the potential of rumen contents is very constant and is stabilized by factors other than the reducing substances present. It is commonly agreed that many facultative organisms are present in the rumen and it is upon the presence of these organisms that an explanation of the stability may be based. The facultative organisms simply utilize the highly oxidative compounds that enter the rumen as hydrogen acceptors and, thereby, maintain the potential at a low level so that the anaerobic population can continue to metabolize. This function is a symptom of the dynamic equilibrium which exists in the rumen.

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PROTEIN-BOUND IODINE IN THE BLOOD OF DAIRY CATTLE FED A TRACE-MINERALIZED SALT [1]

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SUMMARY

Protein-bound iodine (PBI) was determined on the blood plasma of cows, heifers, and calves fed 1% trace-mineralied salt in the concentrate mixtures. The iodine in the salt was in the form of cuprous iodide and 3,5-diiodosalicylic acid. The PBI of milking cows averaged 15.62 γ per 100 ml. at 30 days postpartum, 13.34 at 150 days, and 10.93 at 270 days. The PBI averaged 24.13 γ per 100 ml. for Holsteins, 19.89 for Brown Swiss, 15.81 for Jerseys, and 15.16 for Guernseys. The range of PBI values for all milking cows was 4.69 to 44.28 γ per 100 ml. Highly significant differences in PBI levels of milking cows were found in respect to stage of lactation, breed, and season of year. The PBI was higher in spring and summer, declining to a low in January. Correlation coefficients between PBI and 4% FCM produced were positive for all breeds and significantly so in most of the comparisons. However, the significance of the correlations is questionable, because of the possible masking by the trace-mineralized salt.

The PBI levels of calves and heifers varied widely, ranging from 2.02 to 46.67 γ per 100 ml. of plasma. With calves and heifers the differences were significant for breed, highly significant for seasons, and nonsignificant for age. In general, the plasma PBI levels decreased with age. No significance relationship was found between PBI and growth as measured by increase in height at withers. The feeding of the trace-mineralized salt containing cuprous iodide and 3,5-diiodosalicylic acid apparently caused the high values found.

It is generally accepted that in the Southern United States the milk production of cows is adversely affected by summer conditions. However, it is not clear just how much decline in production is due to the direct effect of climate on the physiology of the animal, and how much is due to a decrease in forage quality and total feed consumption. There are indications that cows in the southern states show different patterns of growth, production, and reproduction and have smaller thyroid glands than animals of similar genetic background in the North (14). A reduction of thyroid secretion rate is apparently one of the means by which an animal adapts to hot conditions (5).

Long *et al.* (12) have reported an average protein-bound iodine (PBI) of 3.15 γ per 100 ml. of blood serum in dairy and beef animals. The PBI values by breeds were: Jersey, 4.11; Guernsey, 3.51; Brown Swiss, 3.37; Ayrshire, 3.19; Holstein, 2.73; and beef breeds, 2.19 γ per 100 ml. Calves averaged 4.8 γ per 100 ml., cows 3 to 4 yr. of age averaged 3.1, and animals 7 to 8 yr. of age averaged 2.6 γ per 100 ml. Plasma PBI concentration tends to decrease with age (10, 11, 12). Lewis and Ralston (10) reported calves under 48 hr. of age averaged 13.7 γ per 100 ml., whereas those between two days and 12 mo. averaged 7.2 γ per 100 ml. Heifers between 1 and 2 yr. of age averaged 6.2 and older

Received for publication October 12, 1959.

[1] Technical Contribution No. 324, South Carolina Agricultural Experiment Station, Clemson, South Carolina.

[2] Data presented are part of a thesis by the senior author submitted to the Graduate School of Clemson College in partial fulfillment of the requirements for the Degree of Master of Science.

cows 4.6 γ per 100 ml., respectively. Lewis and Ralston (10) found that Jerseys and Holsteins had lower PBI concentrations in November than in June, August, or March. The seasonal variations were statistically significant, however, only for the Holstein cows.

Lennon and Mixner (9) determined PBI values on the blood of 93 Holstein cows and 34 Guernsey cows over a 3-yr. period. No correlations between PBI and measures of lactational performance achieved statistical significance, except for the correlation coefficient of −.18 between PBI and fat per cent in the Holstein data on an among-cows-in-lactation basis.

Asplund *et al.* (1) have reported unusually high PBI values for dairy cattle. Levels of from 15 to 30 γ per 100 ml. were common, and values as high as 75 γ per 100 ml. were obtained. The high levels were seasonal, occurring mostly in the late winter and spring. The rise in PBI paralleled the feeding of grass silage to the dairy herd and was not associated with the feeding of excessive quantities of iodine compounds.

The objective of this research was to determine the levels of PBI in the blood plasma of dairy cattle of various age groups, breeds, and stages of lactation in the Clemson College dairy herd. During this experiment a trace-mineralized salt was fed. As the experiment progressed and high PBI values were being found, the influence of the feeding of trace-mineralized salt was determined (7). The intake of a salt containing cuprous iodide and 3,5-diiodosalicylic acid apparently caused the elevated PBI values. Salt containing KI, fed at the same level, caused no apparent increase of the PBI levels (7).

EXPERIMENTAL PROCEDURE

Data for this study were collected during the period from June, 1958, through April, 1959, on cows and heifers from the Clemson College dairy herd. With exceptions noted, the animals used in this study were fed concentrate mixtures containing 1% trace-mineralized salt analyzing 0.0074% iodine. The iodine in the salt was in the form of cuprous iodide and 3,5-diiodosalicylic acid.

The study was in four parts: (a) Stage-of-lactation comparison, consisting of Holstein and Guernsey cows at each of three stages of lactation, 30 days, 150 days, and 270 days after parturition. Blood samples of the cows were taken three times during the year, in June, October, and January. (b) Season-of-year-and-breed comparison, consisting of Holstein, Brown Swiss, Guernsey, and Jersey cows in a similar stage of lactation, from 2 to 5 mo. after parturition. Plasma PBI analyses were made on the blood of these animals during the month of July, to correspond with the middle of summer, September, the time at which milk production normally drops to its lowest level; January, the start of the upturn in the herd production curve; and April, the period of highest production. (c) Calves-and-heifers comparison, consisting of calves under 3 mo. of age, and heifers of breeding age (approximately 18 mo.). Blood samples were taken during the months of June, September, December, and March. (d) Three-and-six-months-old-calves comparison, consisting of 19 Holstein, five Brown Swiss, and 12 Guernsey calves, sampled at 3 and again at 6 mo. of age. Additional 3-mo. calves were sampled to give values during 10 mo. of the year.

Blood samples were collected from the animal's jugular vein, using sodium citrate as the anticoagulant. The blood samples were centrifuged shortly after collection and duplicate determinations of plasma PBI begun immediately by the modified (8) method of Brown *et al.* (3). The procedures for statistical analyses used in the interpretation of data were taken from Cochran and Cox (4).

RESULTS AND DISCUSSION

Stage of lactation. A total of 72 samples of cows' blood was analyzed in duplicate during June, October, and January, to compare the level of plasma PBI with milk production at 30, 150, and 270 days after parturition. The data are presented in Table 1. PBI values decreased in most instances as lactation progressed, averaging 15.62, 13.34, and 10.93 γ per 100 ml., respectively. Values also tended to decrease from a high in June to a low in January, averaging 14.42, 13.29, and 12.18 γ per 100 ml., respectively. Over-all, the Holsteins averaged 15.08 and the Guernseys, 11.51 γ per 100 ml. The PBI averages by breeds and the average consumption of iodine per day from the concentrate mixture are presented in Table 2.

An analysis of variance on these PBI data indicates a highly significant difference between Holstein and Guernsey breeds and between stages of lactation (days). Seasonal difference was not significant in the three seasons studied. The coefficient of correlation between plasma PBI and 4% FCM for the ten-day period in which the PBI was determined indicates a highly significant relationship of .61 for Guernseys and .73 for Holsteins. Animals in this group received

TABLE 1

Mean PBI values of blood plasma from cows to compare stage of lactation and milk production and with season of year and breed

Breed	June	July	September	October	January	April	Average
			(Expressed as γ per 100 ml.)				
			30 days postpartum				
G	14.24(4)[a]			11.53(4)	14.56(4)		13.44
H	19.69(4)			17.96(4)	15.76(4)		17.80
Av.	16.96			14.75	15.16		15.62
			150 days postpartum				
G	13.81(4)			10.46(4)	8.80(4)		11.02
H	16.41(4)			17.96(4)	12.59(4)		15.65
Av.	15.11			14.21	10.70		13.34
			270 days postpartum				
G	10.61(4)			11.10(4)	8.49(4)		10.07
H	11.79(4)			10.72(4)	12.86(4)		11.79
Av.	11.20			10.91	10.68		10.93
			Seasons of year and breed[b]				
G		15.01(6)	12.56(7)		13.14(7)	19.93(6)	14.98
H		28.17(7)	21.41(8)		18.91(7)	28.01(6)	23.89
BS		21.38(6)	18.02(7)		13.94(6)	26.23(6)	19.82
J		14.79(6)	14.34(3)		14.82(4)	19.19(6)	16.14
Av.		20.17	17.13		15.32	23.34	18.71

[a] Number of cows in each group average.
[b] Cows were 2 to 5 mo. postpartum.

TABLE 2

Summary of mean plasma PBI values for Holsteins and Guernseys at three stages of lactation by days postpartum and three seasons of year and the iodine consumed per cow daily from the trace-mineralized salt

Month	Holsteins			Guernseys		
	30	150	270	30	150	270
			(γ per 100 ml.)			
June	19.69	16.41	11.79	14.24	13.81	10.61
October	17.96	17.96	10.72	11.53	10.46	11.10
January	15.76	12.59	12.86	14.56	8.80	8.49
Av.	17.80	15.65	11.79	13.44	11.02	10.07
			Av. mg. of salt iodine intake per day			
	4.47	3.92	2.10	3.55	2.94	2.10

in their concentrate 2.10 to 4.47 mg. of iodine per cow daily from the trace-mineralized salt in the concentrate mix (Table 2).

Season of year and breed. The season of year data and breed comparisons are presented in Table 1 and summarized in Table 3, and include PBI determinations on the plasma of 25 Brown Swiss, 28 Holstein, 26 Guernsey, and 19 Jersey cows that were 2 to 5 mo. postpartum during one of the months of July, September, January, or April. PBI values generally decreased from a high in July to a low in January, and then in April were back to the original levels or above. Breed levels ranged downward as follows: Holstein, Brown Swiss, Jersey, and Guernsey. An analysis of variance of the PBI values indicates a highly significant difference between breeds and between seasons of the year. Although the relationships may be upset by feeding of the trace-mineralized salt, correlations are presented as information. The coefficient of correlation between PBI and 4% FCM was positive for all breeds, and highly significant for Brown Swiss (0.60), Guernseys (0.51), and Jerseys (0.66). The correlation for Holsteins (0.33) was not significant. The iodine in the ration supplied by trace-mineralized salt ranged from 3.53 to 5.20 mg. of iodine per cow per day (Table 3). The coefficient of correlation of concentrate consumption and, therefore, the iodine intake, with the PBI levels of the cows' plasma, was .0003, indicating no simple relationship between the two, and may be an expression of genetic differences between animals.

With the exception of West Virginia (1), plasma PBI values on these cows were considerably higher than those previously reported from other stations.

TABLE 3

Summary of mean plasma PBI values for four breeds and four seasons of year and iodine consumed per cow daily from trace-mineralized salt

Month	Holstein	Brown Swiss	Jersey	Guernsey
		(γ per 100 ml.)		
July	28.17	21.38	14.79	15.01
September	21.41	18.02	14.34	12.56
January	18.91	13.94	14.92	13.14
April	28.01	26.23	19.19	19.93
Av.	24.13	19.89	15.81	15.16
		Av. mg. of salt iodine intake per day		
	5.20	4.16	3.53	3.57

TABLE 4

Mean PBI values of blood samples collected from dairy calves and heifers by ages and months of year
(Expressed as γ per 100 ml.)

Breed	June	July	August	September	October	November	December	January	February	March
					Calves under 3 mo.					
G	16.18(3)[a]			7.60(4)			4.81(3)			6.10(3)
H	14.68(6)			9.15(6)			5.78(6)			10.80(6)
BS	14.80(3)			4.33(1)			10.21(2)			4.67(3)
Av.	15.09			8.15			6.32			8.09
					Heifers' breeding age					
G	8.52(3)			4.52(4)			11.76(3)			2.92(3)
H	11.08(6)			5.81(5)			13.91(6)			2.87(6)
BS	13.25(3)			10.18(3)			23.74(3)			2.52(3)
Av.	10.98			6.47			15.83			2.80
					Calves 3 mo.					
G	16.18(3)	10.79(1)	18.92(2)	15.70(2)		12.72(1)	4.81(3)	13.59(1)	9.69(3)	8.31(1)
H	19.13(3)	40.42(3)	17.40(1)	28.19(2)	38.24(1)	15.13(7)	6.62(2)	13.03(2)	18.88(3)	15.77(3)
BS	22.73(1)	43.54(1)	46.47(1)	25.90(1)		12.19(1)		18.21(1)		
Av.	18.38	35.12	25.43	22.74	38.24	14.54	5.53	14.47	14.29	13.91
					Calves 6 mo.					
G				6.39(3)	14.77(1)	13.80(2)	23.77(2)		9.63(1)	6.34(3)
H				5.55(3)	12.17(3)	12.94(1)	42.13(2)	12.26(1)	23.65(7)	11.97(2)
BS				4.69(1)	22.12(1)	2.26(1)	22.68(1)			9.69(1)
Av.				5.79	14.68	10.70	30.90	12.26	21.90	8.78

[a] ( ) Number of animals in each group average.

Other workers (1, 2, 6, 10) have reported lower values for the fall and winter months, corresponding with the results obtained in this study.

Calves and heifers. Plasma PBI values comparing calves under 3 mo. of age to heifers of breeding age (Table 4) showed considerable variation, especially between months, and few clear patterns were noted. The calves in general had higher values than the heifers. Breed levels ranged from high to low as follows: Brown Swiss, Holstein, and Guernsey, but with no great differences. An analysis of variance of the PBI values indicates a highly significant difference between seasons and interactions of age and season. Also, significant differences between breeds and interactions of age and breed, and breed and season were found. Very low values were found for 12 breeding age heifers during March. This group of heifers did not receive trace-mineralized salt in the concentrate being fed, and that probably was the reason for the low values.

Three- and six-mo.-old calves. The data comparing the 3-mo.-old calves to the same calves at 6 mo. old indicate no significant differences between breed or age as calculated by analysis of variance. Results again showed considerable variation. Six-mo.-old calves tended to have lower plasma PBI values than 3-mo.-old calves. A coefficient of correlation between average plasma PBI values for 3- and 6-mo.-old calves, and gain in height of withers during the 3 and 6 mo. period was run, to determine if there was a correlation between growth rate and PBI level. This was run separately on 14 Holsteins and nine Guernseys in the group, as well as 27 grouped animals. No significant relationship between the two was found in any case. The lack of significance may be due to the variability of the values.

Other stations (10, 13) have reported lower plasma PBI values in mature animals than in young calves. The PBI of the calves in this study increased up to about 3 mo. of age, possibly due to an increased intake of trace-mineralized salt, and were higher than in the milking cows. In general, and possibly for the same reason, the heifers at breeding age were lower in plasma PBI than the milking cows.

It was apparent from the results being obtained that there was some factor or factors causing these unusually high plasma PBI values. The reagents being used and the procedures being followed for determinations were checked closely and no error was noted. Four blood samples were taken, the plasma separated, and analyzed for PBI in this laboratory, and duplicate plasma samples were sent to another laboratory which was using the same analytical method. The analyses from the two laboratories checked closely.

The feeding of trace-mineralized salt containing cuprous iodide and 3,5-diiodosalicyclic acid apparently caused the higher PBI values of blood plasma (7) because, after removal of this salt from the ration or replacement by KI-salt, the plasma PBI values fell within what is considered a normal range.

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VARIABLES AFFECTING RATIO FACTORS FOR ESTIMATING 305-DAY PRODUCTION FROM PART LACTATIONS [1]

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SUMMARY

Complete lactations for 12,561 Holsteins, 2,262 Guernseys, 990 Jerseys, and 459 Brown Swiss recorded in Michigan DHIA-IBM from June 1954 through July 1957 were analyzed to ascertain the relative effects of breed, herd, age, parity, and season of freshening on the relationship of total to part milk production, and then were used to derive ratio factors for extending partial records to 305 days.

The ratio of total milk produced on ten monthly test days to milk produced on each test day was used to measure the relationship between total and part production. Components of variance of ratios indicated that lactation number had a larger influence on the total to part relationship than did age at freshening. Season of freshening also exerted an influence, but to a slightly less degree than either lactation number or age, while the effect of herd was small and unimportant. Differences between breeds existed both in the components of variance and in the total to part relationship.

Ratio factors for extending records from each of ten monthly test days and from cumulative test-day production were computed for different ages, lactation numbers, and seasons of freshening for each of the four breeds. Only small differences exist between the factors which adjust for ages and those which adjust for lactation number, indicating that either set of factors should adequately extend incomplete records. However, factors based on age are more useful in extending those records in which age at freshening and lactation number do not coincide. In practice breed, age, and season of freshening should be considered in extending partial records to 305 days.

More than one-fifth of the lactations started in DHIA are not completed because the cows die or are sold (1, 7). Seldom are the records terminated early in lactation used in programs to improve dairy cattle, and what information they contain is lost or ignored. One method of salvaging the information which is lost when a cow leaves the herd prior to completing a lactation is to extend the incomplete record to estimate from its production before leaving the herd what the cow would have produced had it remained.

The most important use for extended incomplete records is to make possible including in a sire's progeny test a record for every daughter that comes into production. This would help to standardize sire proofs by compensating for selective removal of poor producers from the herd prior to completion of the first lactation. Extended incomplete records can also be used to project what a cow will produce in a lactation while that lactation is still in progress. This early information would facilitate selection prior to the completion of a lactation. At present, selection of a bull on the performance of his offspring is delayed until several of his daughters have completed at least one lactation. Projection of records still in progress could reduce the time needed to prove a sire by up to 9 mo.

Several workers (2–5, 8–11) have reported multiplicative and regression factors for estimating complete lactation yield from various portions of the lac-

Received for publication October 5, 1959.

[1] Journal Article No. 2502. Michigan Agricultural Experiment Station.

tation. Their studies have shown that several nongenetic variables should be adjusted for in extending incomplete lactations. Extension factors which will correct for the effects of all of these variables are not available, nor are they practical. Nevertheless, the variables which contribute a relatively larger portion of the total variation in the total to part relationship do need to be corrected for in extending short-time records.

This study was initiated to investigate the relative effects of breed, herd, age, parity, and season of freshening on the relationship between total milk produced in 305 days and milk produced in each month, and to develop factors, based on these findings, for extending incomplete lactations to 305 days.

SOURCE OF DATA

The data were 16,272 complete lactation records from four major dairy breeds obtained from Michigan DHIA-IBM for the period June 1954 through July 1957. Included were 12,561 Holstein, 2,262 Guernsey, 990 Jersey, and 459 Brown Swiss lactations. Each of these records conformed to the following specifications: (a) 2-time milking, (b) less than 50 days production calculated from a single test day, and (c) ten consecutive monthly tests. All records were terminated at 10 mo. Production on test day was recorded to the nearest one-tenth pound of milk, and the nearest one-hundredth pound of fat.

METHODS AND RESULTS

The data were analyzed separately for each breed. In order to consider the postulated environmental influences, the data were grouped into four seasons of freshening (October-December, January-March, April-June, July-September), four ages (under 36 mo., 36-47 mo., 48-59 mo., and 60 mo. and over), and three lactation numbers (first, second, and all subsequent lactations). Because of the disproportionate distribution of data in this study, ages and lactation numbers were studied on a within-herd basis.

The ratio of total milk produced on ten monthly test days to milk produced on each test day was used to measure the relationship between total and part production. The ratios of total to part production were calculated to the nearest one-hundredth pound.

The components of variance of the ratios were estimated, using the first method described by Henderson (6). In using this method an assumption is made that all elements of the model are uncorrelated. A positive correlation between age and lactation number is known to exist; however, since in this study only the larger sources of variation are being sought, what bias does exist is not as important as if precise estimates were desired. Henderson (6) presents another method for estimating components of variance which yields unbiased estimates, but it is computationally prohibitive. The results of the analysis of variance are presented in Tables 1, 2, 3, and 4.

All components vary considerably between months of the lactation and between breeds. A few extreme departures from the general trend occur; however, these appear to be sampling rather than identified sources of variation. Residual

TABLE 1

Components of variance for the ratio of total production to monthly production—Brown Swiss

	Month																	
	1		2		3		4		5		6		7		8		9		10	
Source	Var.	%	Var.	%	Var.	%	Var.	%	Var.	%	Var.	%	Var.	%	Var.	%	Var.	%	Var.	%
Age	2.53	27	1.34	21	−.19	0	−.01	0	−.04	0	−.17	0	.28	8	−.20	0	.90	5	12.07	6
Lactation	1.27	13	.95	15	.13	8	−.06	0	.03	1	−.26	0	.27	8	.69	11	3.72	19	32.94	18
Herd	−.41	0	−.34	0	−.01	0	−.15	8	.04	2	.14	6	−.21	0	−.02	0	−1.08	0	−16.84	0
Season	.02	0	.15	2	.24	15	.32	18	.35	20	.28	11	.64	18	1.00	16	2.96	15	7.12	4
SH	.55	6	.43	7	.06	4	−.51	0	.12	7	−.18	0	.44	12	.20	3	1.64	8	42.58	23
SA	−1.23	0	−1.03	0	.18	11	.25	14	.05	3	−.09	0	−.05	0	−.15	0	.63	3	−7.18	0
SL	−.42	0	−.24	0	−.12	0	.37	21	−.13	0	.44	17	−.39	0	.21	3	−3.14	0	−38.24	0
AL	−3.21	0	−1.87	0	.40	25	.18	10	.13	8	.27	11	−.01	0	−.17	0	−1.36	0	−20.81	0
Residual	5.16	54	3.64	55	.60	37	.54	29	1.02	59	1.40	55	1.90	54	3.99	67	9.66	50	93.51	49
Total[a]	9.52		6.51		1.62		1.81		1.75		2.53		3.55		6.09		19.50		188.22	

[a] Negative components were considered zero for computing totals and percentages.

TABLE 2

Components of variance for the ratio of total production to monthly production—Guernsey

	Month																	
	1		2		3		4		5		6		7		8		9		10	
Source	Var.	%	Var.	%	Var.	%	Var.	%	Var.	%	Var.	%	Var.	%	Var.	%	Var.	%	Var.	%
Age	.23	10	.18	12	−.57	0	.84	28	.22	8	0	0	−.26	0	2.08	16	4.70	12	15.51	8
Lactation	.39	16	.17	11	−.11	0	−.89	0	.19	7	.04	1	.21	5	1.30	10	6.75	17	3.75	2
Herd	−.02	0	.02	1	.02	1	−.02	0	−.08	0	.02	1	.05	1	.14	1	−.26	0	−3.52	0
Season	.13	5	.17	11	.25	13	.37	12	.43	16	.05	2	.82	19	2.17	17	5.64	14	16.34	8
SH	.11	5	−.06	0	−.04	0	.04	1	.36	13	.20	7	.08	2	.17	1	1.04	3	19.12	9
SA	−.21	0	−.13	0	.39	21	.01	0	−.11	0	.55	18	−.20	0	.44	3	2.23	6	−.43	0
SL	.17	7	.16	10	.06	3	.04	1	−.34	0	−.48	0	.36	8	−.26	0	−5.04	0	−24.04	0
AL	−.32	0	−.14	0	.99	53	.69	23	−.34	0	−.21	0	.12	3	−2.37	0	−6.46	0	14.86	7
Residual	1.40	57	.82	55	.14	8	.97	35	1.50	56	2.14	71	2.65	62	6.62	52	18.99	48	133.95	66
Total[a]	2.44		1.51		1.85		2.96		2.70		3.00		4.29		12.92		39.34		203.54	

[a] Negative components were considered zero for computing totals and percentages.

TABLE 3

Components of variance for the ratio of total production to monthly production—Holstein

Source	Month 1 Var.	%	2 Var.	%	3 Var.	%	4 Var.	%	5 Var.	%	6 Var.	%	7 Var.	%	8 Var.	%	9 Var.	%	10 Var.	%
Age	.19	7	.04	3	.46	20	.23	13	.56	17	.42	14	−.46	0	.09	1	2.27	9	−43.55	0
Lactation	.50	18	.22	15	−.04	0	.23	13	.20	6	.27	9	1.69	21	.86	13	4.16	17	17.32	6
Herd	−.13	0	−.09	0	−.15	0	−.16	0	−.15	0	−.22	0	−.43	0	−.28	0	−1.06	0	3.67	1
Season	.13	5	.10	7	.13	6	.17	9	.16	5	.18	6	.37	5	.78	12	1.92	8	6.30	2
SH	.18	6	.03	2	.20	9	.25	14	.24	8	.38	12	1.17	14	.65	10	1.41	6	−1.50	0
SA	.19	7	.02	1	−.44	0	−.06	0	−.44	0	−.18	0	3.82	46	.31	5	1.14	5	76.88	28
SL	−.22	0	.13	9	.19	8	−.13	0	.01	0	−.24	0	−2.89	0	−.48	0	−1.94	0	−37.38	0
AL	−.30	0	.07	5	−.10	0	−.21	0	−.53	0	−.33	0	−.98	0	.31	5	−2.06	0	38.71	14
Residual	1.61	57	.84	58	1.33	57	.91	51	1.94	64	1.82	59	1.10	14	3.58	54	13.21	55	130.76	49
Total[a]	2.80		1.45		2.32		1.80		3.06		3.05		8.15		6.59		24.11		273.64	

[a] Negative components were considered zero for computing totals and percentages.

TABLE 4

Components of variance for the ratio of total production to monthly production—Jersey

Source	Month 1 Var.	%	2 Var.	%	3 Var.	%	4 Var.	%	5 Var.	%	6 Var.	%	7 Var.	%	8 Var.	%	9 Var.	%	10 Var.	%
Age	.15	4	0	0	.24	15	.13	8	−.53	0	.39	11	.47	6	4.21	16	4.28	14	11.46	4
Lactation	.58	17	.08	6	.14	9	−.01	0	−.03	0	.48	13	.74	10	1.13	4	1.96	7	47.22	17
Herd	.09	3	.08	6	.09	5	.11	7	.07	2	−.07	0	28	4	3.38	13	4.01	14	3.42	1
Season	.08	2	.18	14	.21	13	.27	16	.39	13	.53	14	1.21	17	1.70	7	5.63	19	14.81	5
SH	.19	5	0	0	.04	3	.06	4	.23	8	.21	6	.46	6	−.89	0	−.16	0	30.50	11
SA	1.15	33	−.12	0	−.09	0	−.16	0	.38	13	.02	0	−.56	0	−5.13	0	1.26	4	20.45	7
SL	−.85	0	.11	8	0	0	.02	1	−.58	0	−.16	0	−.13	0	.97	4	−1.44	0	−34.13	0
AL	−.55	0	.06	4	−.19	0	−.01	0	.70	23	−.75	0	−.79	0	−5.19	0	−4.76	0	−61.14	0
Residual	1.28	36	.85	62	.86	55	1.11	64	1.25	41	2.09	56	4.08	57	14.41	56	12.44	42	157.62	55
Total[a]	3.52		1.36		1.59		1.70		3.02		3.72		7.24		25.80		29.59		285.47	

[a] Negative components were considered zero for computing totals and percentages.

differences are the largest. Since this portion of the variance will not be adjusted for, a few of the extended individual lactations may vary considerably from actual, but most of the predicted records should be close to actual production. Lactation number contributes a larger portion of the variation in the relationship of total to part than does age. Differences due to season of freshening contribute a relatively large portion of the variance. There are some indications of breed differences in this component, with Holsteins being least influenced by season of freshening. The variance due to season of freshening increases with each succeeding month in the lactation, with the largest increase occurring during the tenth month. Although the actual variance due to season of freshening increases greatly in the tenth month, variance attributable to the other sources increases even more, so that the effect of season relative to other sources decreases during the tenth month. Differences between herds account for almost no variation in the relationship of total to part production. Season-herd interaction contributes a relatively large portion of the variation, particularly in Holsteins. This indicates that although herds do not vary appreciably in their effect on the total to part relationship, there is a difference between herds as to the influence season of freshening has upon this relationship. No other interactions are important.

Noncumulative ratio factors for extending records from each of ten individual monthly tests were computed for different ages, lactation numbers, and seasons of freshening for each of the four breeds by averaging the ratio of total milk produced on ten test days to milk production for each test day for all records in that particular group. Separate factors were computed for first, second, and all later lactations; however, those for second lactations were so similar to those for lactations three and above that the second lactations were incorporated with later records and one set of combined factors was obtained. This agrees with the earlier reports of Madden and coworkers (9, 10, 11), who found that differences existed between factors for heifers and older cows, but that one set of factors was satisfactory for all older ages. Because of the similarity of these results, factors were computed for each of the two age groups, under 36 mo. and 36 mo. and over. Noncumulative ratio factors by breed, age, and season of freshening are presented in Table 5.

An analysis of the components of variance for the ratio of total production to cumulative production was not made, although this possibly would have provided a sounder basis for determining the sources of variation to be considered in developing cumulative ratio factors. Cumulative factors were obtained by adding the reciprocals of noncumulative factors and then reciprocating. Table 6 presents cumulative factors which will adjust for breed, age, and season of freshening, and Table 7 presents cumulative factors which will adjust for breed, parity, and season of freshening. Factors which adjust for any of these variables, either individually or in a combination of two, can be obtained from Tables 6 or 7 by summing over the variables not to be included and weighting each factor according to the number of records used to obtain that factor.

TABLE 5
Noncumulative ratio factors by breed, age, and season of freshening

Test Day	Brown Swiss < 36 Nov.-Apr. (48)[a]	Brown Swiss < 36 May-Oct. (55)	Brown Swiss ⩾ 36 Nov.-Apr. (159)	Brown Swiss ⩾ 36 May-Oct. (197)	Guernsey < 36 Nov.-Apr. (249)	Guernsey < 36 May-Oct. (428)	Guernsey ⩾ 36 Nov.-Apr. (709)	Guernsey ⩾ 36 May-Oct. (876)
1	9.42	8.32	8.02	7.55	8.06	7.83	7.36	6.83
2	9.08	8.51	7.96	7.74	8.00	8.22	7.38	7.34
3	8.98	9.19	8.27	8.36	8.51	9.05	8.10	8.37
4	9.18	9.84	8.80	9.30	9.24	9.84	8.84	9.55
5	9.40	10.19	9.29	10.30	9.68	10.57	9.43	10.59
6	9.88	10.85	9.92	11.06	10.22	11.06	10.32	11.59
7	10.29	11.11	11.08	11.78	11.08	11.47	11.56	12.27
8	11.59	11.20	13.02	12.31	12.47	11.27	13.84	12.94
9	13.03	11.40	15.44	13.73	14.12	11.87	17.47	14.32
10	17.45	13.47	20.90	17.72	17.19	13.43	25.39	18.94

Test Day	Holstein < 36 Nov.-Apr. (1,326)	Holstein < 36 May-Oct. (2,613)	Holstein ⩾ 36 Nov.-Apr. (3,632)	Holstein ⩾ 36 May-Oct. (4,990)	Jersey < 36 Nov.-Apr. (102)	Jersey < 36 May-Oct. (224)	Jersey ⩾ 36 Nov.-Apr. (266)	Jersey ⩾ 36 May-Oct. (398)
1	8.59	8.22	7.81	7.33	7.95	7.72	7.32	6.92
2	8.31	8.40	7.62	7.56	7.75	8.03	7.59	7.22
3	8.69	9.10	8.13	8.42	8.49	8.90	8.20	8.24
4	9.17	9.63	8.81	9.26	9.27	9.79	9.01	9.46
5	9.59	10.16	9.44	10.10	9.52	10.64	9.61	10.62
6	10.02	10.65	10.19	10.92	10.37	11.29	10.12	11.62
7	10.64	11.10	11.76	11.73	11.09	11.69	11.09	12.49
8	11.72	11.43	13.31	12.52	12.47	11.53	13.61	13.36
9	13.50	11.97	16.03	14.50	14.70	12.09	16.91	14.62
10	17.14	14.49	23.94	20.36	17.41	14.13	23.81	19.13

[a] Numbers of records averaged to obtain the ratio factors.

DISCUSSION

Variables affecting relationship of total to part production. From a standpoint of ease and efficiency, one set of general factors for extending all part-time records is optimum. Unfortunately, the solution is not that simple. Several workers have shown that there are environmental influences which should be adjusted for in extending incomplete records. One of the purposes of this study was to ascertain the relative influence of five of these variables on the relationship between total milk production and production during various portions of the lactation, in order that factors may be obtained which adjust for the most important variables.

Even though age and lactation number are, to a large extent, merely different measures of the same variable, they are not entirely synonymous. They were studied concurrently to determine if one was exerting a larger influence on the relationship of total to part production than was the other. The result of this study supports earlier reports (3, 5, 8, 9, 10, 11), that the total to part relationship differs between heifers and older cows and, in addition, indicates that

TABLE 6
Cumulative ratio factors by breed, age, and season of freshening

Test Day	Brown Swiss				Guernsey			
	< 36		⩾ 36		< 36		⩾ 36	
	Nov.-Apr. (48)[a]	May-Oct. (55)	Nov.-Apr. (159)	May-Oct. (197)	Nov.-Apr. (249)	May-Oct. (428)	Nov.-Apr. (709)	May-Oct. (876)
1	9.42	8.32	8.02	7.55	8.06	7.83	7.36	6.83
2	4.62	4.21	4.00	3.82	4.01	4.01	3.68	3.54
3	3.05	2.89	2.69	2.62	2.73	2.78	2.53	2.49
4	2.29	2.23	2.06	2.05	2.11	2.17	1.97	1.97
5	1.84	1.83	1.69	1.71	1.73	1.80	1.63	1.66
6	1.55	1.57	1.44	1.48	1.48	1.55	1.41	1.45
7	1.35	1.37	1.28	1.31	1.31	1.36	1.25	1.30
8	1.21	1.22	1.16	1.19	1.18	1.22	1.15	1.18
9	1.11	1.10	1.08	1.09	1.09	1.10	1.08	1.09

Test Day	Holstein				Jersey			
	< 36		⩾ 36		< 36		⩾ 36	
	Nov.-Apr. (1,326)	May-Oct. (2,613)	Nov.-Apr. (3,632)	May-Oct. (4,990)	Nov.-Apr. (102)	May-Oct. (224)	Nov.-Apr. (266)	May-Oct. (398)
1	8.59	8.22	7.81	7.33	7.95	7.72	7.32	6.92
2	4.22	4.15	3.86	3.72	3.92	3.94	3.73	3.53
3	2.84	2.85	2.62	2.58	2.68	2.73	2.56	2.47
4	2.17	2.20	2.02	2.02	2.08	2.13	1.99	1.96
5	1.77	1.81	1.66	1.68	1.71	1.78	1.65	1.65
6	1.50	1.55	1.43	1.46	1.47	1.54	1.42	1.45
7	1.32	1.36	1.27	1.30	1.30	1.35	1.26	1.30
8	1.18	1.21	1.16	1.17	1.17	1.21	1.15	1.18
9	1.09	1.10	1.08	1.09	1.09	1.10	1.08	1.09

[a] Numbers of records averaged to obtain the ratio factors.

lactation number accounts for more of the variability in the total to part relationship than does age alone.

The ratio factors themselves indicate no practical differences between factors for age and lactation number. The differences between noncumulative age and lactation factors are small and unimportant, while those of cumulative factors are small for the first four cumulative months and almost nonexistent thereafter. Cumulative Holstein factors for both ages and lactations are similar to the cumulative age factors obtained by Madden *et al.* (11) from Holstein HIR data.

From a practical standpoint, extension factors based on age should be used to extend first lactation records initiated after 36 mo. of age, since factors based on parity will overestimate production and favor an undesirable managemental situation. Factors for age should also be used to extend second lactation records started prior to 36 mo., since using factors for lactation in this case will underestimate production and penalize a desirable breeding practice. The remaining 90% of the time either age or lactation factors will work equally well. In practice, all incomplete records can be extended by factors for age irrespective of lactation number, whereas factors for lactation should be used in combination with age 10% of the time. In view of this, factors based on age are preferable to factors based on parity for extending incomplete records.

TABLE 7
Cumulative ratio factors by breed, lactation number, and season of freshening

Test Day	Brown Swiss				Guernsey			
	Lactation 1		Lactation ⩾ 2		Lactation 1		Lactation ⩾ 2	
	Nov.-Apr. (53)[a]	May-Oct. (60)	Nov.-Apr. (154)	May-Oct. (192)	Nov.-Apr. (261)	May-Oct. (436)	Nov.-Apr. (697)	May-Oct. (868)
1	9.35	8.36	8.00	7.51	8.11	7.89	7.33	6.79
2	4.61	4.23	3.98	3.80	4.03	4.04	3.68	3.52
3	3.05	2.90	2.68	2.61	2.74	2.79	2.53	2.47
4	2.29	2.24	2.06	2.04	2.11	2.18	1.96	1.96
5	1.84	1.84	1.68	1.70	1.73	1.81	1.63	1.66
6	1.55	1.57	1.44	1.47	1.48	1.55	1.40	1.45
7	1.34	1.38	1.28	1.31	1.31	1.37	1.25	1.30
8	1.20	1.23	1.16	1.18	1.18	1.22	1.15	1.18
9	1.10	1.11	1.08	1.09	1.09	1.10	1.08	1.09

Test Day	Holstein				Jersey			
	Lactation 1		Lactation ⩾ 2		Lactation 1		Lactation ⩾ 2	
	Nov.-Apr. (1,412)	May-Oct. (2,698)	Nov.-Apr. (3,546)	May-Oct. (4,905)	Nov.-Apr. (97)	May-Oct. (208)	Nov.-Apr. (271)	May-Oct. (414)
1	8.64	8.28	7.78	7.27	8.03	7.69	7.30	6.96
2	4.24	4.18	3.84	3.70	3.97	3.93	3.71	3.55
3	2.85	2.87	2.61	2.57	2.71	2.73	2.55	2.48
4	2.18	2.21	2.01	2.01	2.10	2.13	1.99	1.97
5	1.77	1.82	1.66	1.68	1.72	1.78	1.65	1.66
6	1.51	1.55	1.43	1.45	1.47	1.54	1.42	1.45
7	1.32	1.36	1.27	1.29	1.30	1.36	1.26	1.30
8	1.19	1.21	1.15	1.17	1.18	1.22	1.15	1.18
9	1.09	1.10	1.08	1.08	1.09	1.11	1.08	1.10

[a] Numbers of records averaged to obtain the ratio factors.

The observation that season of freshening is almost as important a source of variation in the whole to part relationship as is age is not in complete agreement with the literature. Eldridge and Atkeson (3) and Fritz (4), using methods of regression, considered the effect of season of freshening on this relationship, but concluded that it was insignificant. On the other hand, Kendrick (8) concluded that season of freshening should be considered for the first 6 mo. in extending cumulative short-time Ayrshire records. In this study, the large differences between factors for each of the different seasons, particularly for the first few cumulative months, indicate that season of freshening does influence the total to part relationship and should be adjusted for in extending records.

The results of the analysis of the components of variance show that herd-to-herd differences do not influence the total to part relationship for milk production, and, therefore, separate extension factors are not required for each herd. This is in complete agreement with the observations made by Fritz (4).

The average ratio factors differ between breeds. Holstein and Brown Swiss factors tend to be alike, while Guernsey factors resemble Jersey factors even more closely. Brown Swiss factors, when applied to cumulative production for any

of the other breeds, will overestimate 305-day production. On the same basis, Holstein factors will overestimate Guernsey and Jersey production, whereas Guernsey factors overestimate Jersey production for the first lactation, but underestimate it for all later lactations. These overestimates in all cases are larger during early lactation, decreasing as production for each succeeding month is added to the cumulative total, and becoming almost negligible after the eighth cumulative month.

In view of the results obtained and the points discussed in preceding paragraphs, it is recommended that age, breed, and season of freshening be taken into consideration in extending part records to a 305-day basis.

Deriving extension factors. Basically, the literature reports two methods for deriving factors for estimating total lactation production from either a single test or from cumulative production. The simplest method is to obtain the ratio of total production to part production. This ratio when multiplied by actual production, equals estimated 305-day production. The other method is to obtain the regression of total production on partial production. Both linear and quadratic regression equations have been used, but Harvey (5) found the curvilinearity of accumulated production and stage of lactation to be small enough that the linear regression equation will provide a satisfactory means of extending part production.

The choice between the two methods depends on the purpose for which extended records are to be used, the ease and simplicity of use, and the comparative accuracy of the methods. The ratio method is by far the simplest and easiest to derive, to use, and to understand. The comparative accuracy of the two methods has been reported previously (5, 10, 11). The ratio method appears to underestimate total production of low-producing cows and overestimate total production of high-producing cows, since it takes into account only the incompleteness of the lactation. The regression method corrects for this incompleteness as well as for the incomplete repeatability of parts of the lactation. Madden *et al.* (11) have pointed out that the variation in total production estimated by ratio is more nearly like the variation in actual total production; whereas, total production estimated by regression is less variable than actual production. If extended part records are used for culling females within a herd or for proving sires, particularly in artificial insemination, extending by means of the ratio method should result in culling the same females or in selecting the same sires as extending by regression, since extending by ratio does not change the order of records but merely spreads them out. Since records extended by regression differ less than actual, this tendency to group the records more closely about the mean may make selection decisions more difficult.

Use of the factors. Two basic types of ratio factors have been presented, cumulative and noncumulative. The cumulative ratio factors are more useful and more widely applicable, since they utilize all of the available test-day information. Since most testing programs report total production to date, an even more practical means of using cumulative extension factors would be to interpolate them, so that cumulative production for any number of days could be extended to 305 days by multiplication by a single factor.

Uninterpolated cumulative factors may be used in cases where only the production for several test days is known. Cumulative test day production is multiplied by 30.5, the average number of days in a month, to give cumulative monthly production, which is then multiplied by the factor for the number of test days involved to obtain estimated 305-day production. Noncumulative factors will be used only when cumulative production is not available. To use these factors, multiply individual test-day production by 30.5 to get monthly production, which is multiplied by the factor for that particular month to obtain estimated 305-day production.

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A COMPARISON OF DAIRY SIRE PROGENY TESTS MADE AT SPECIAL DANISH TESTING STATIONS WITH TESTS MADE IN FARMER HERDS

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SUMMARY

The data consisted of the first-lactation milk and butterfat records of 5,454 daughters of 305 Red Danish Milkrace sires tested at the Danish bull testing stations and the first test-year milk and fat records of 3,270 daughters of 110 of these same sires tested in farmer herds. All records were expressed in kilograms.

For the test station data, the components of variance associated with differences between sires within stations and years were 229 for butterfat and 107,652 for milk. The corresponding components for field data after removing herd differences were 71 and 40,540. After expressing each daughter's record as a deviation from its contemporary herd average, the corresponding components were 53 and 32,125. The within-sire components for butterfat were 1,280, 970, and 916, respectively, for the three methods, and 541,696, 513,697, and 527,477, respectively, for milk. The heritabilities resulting from the three methods, assuming that the sire component is wholly genetic, are 0.66, 0.29, and 0.23 for milk and 0.61, 0.27, and 0.22 for butterfat, respectively. One might expect the estimates of heritability obtained from test station data to be somewhat higher than those obtained from field data, but not nearly so much higher as was found in this study. It is concluded that the estimates based on test station data are higher mainly because environmental differences at the same test station in the same year are confounded with progeny groups, thus putting much environmental variance in the sire components and increasing them more than the within-sire components.

The estimates of the genetic correlation between station tests and field tests were 0.68 for milk and 0.75 for butterfat. On the other hand, the genetic correlations between independent field tests were 0.94 and 0.92 for milk and butterfat, respectively. This suggests that there is either a large interaction between sires and level of management or that the between-sire components from test station data are inflated with environmental differences. The latter cause is a more logical explanation, as the present data and previous data indicate that the interaction between sires and herds is small.

For a constant number of daughters per sire of five or more, the regression of a future daughter in farmer herds on a test in farmer herds exceeds the regression of a future daughter in farmer herds on a test at the test stations. This is true for both milk and butterfat. As the number of daughters per sire increases, the regression based on a field test becomes increasingly larger than the regression based on station tests. For example, for ten daughters the former is 1.44 and 1.48 times the latter for milk and butterfat, respectively; whereas, for 20 daughters the former is 1.73 and 1.75 times the latter for milk and butterfat, respectively.

The expected genetic superiority of future daughters, in farmer herds, of sires chosen on a basis of progeny tests is $b_{x'x}\ (z/b)\ \sigma_x$ for station tests and $b_{x'y}\ (z/b)\ \sigma_y$ for field tests. Assuming that the same percentage of sires is saved as a result of each system of testing, field tests and station tests can be compared. In the case of milk, if the number of daughters per sire is seven or more, then the expected genetic superiority resulting from selection based on field tests is greater than that resulting from station tests. If the number is less than seven, then the reverse is true. In the case of butterfat production, selection on field tests is superior to selection on station tests if the number of daughters is 15 or more. If the number is less than 15, then the reverse is true. As the numbers increase from seven and 15, the superiority of the field test increases for both milk and butterfat. For 20 daughters per sire, the expected genetic improvement from selection

Received for publication October 9, 1959.

based on field tests is 1.14 and 1.02 times that resulting from station tests for milk and butterfat, respectively.

In general, the field tests seem to be superior to station tests if the number of daughters per sire is 15 or more. This superiority increases as the number of daughters per sire increases. In areas where a high percentage of herds is on test there appears to be little need for special dairy sire progeny-testing stations.

Assuming that the environmental differences confounded with sires can be controlled by proper experimental design and station operation, and that the expense of operating stations need not necessarily be high, the most serious limitation of test stations is the number of sires that can be tested. To make the expected genetic gain from selection based on tests at special test stations higher than that now expected, more sires should be tested with fewer daughters per sire.

The yearly records of all tested cows in Denmark have been collected by the Agricultural Societies since 1912. These records have been the basis for progeny-testing dairy sires and have been important to Danish cattle breeders. In 1945, the first dairy sire progeny-testing stations in Denmark were established and from 1947 annual reports, Afkomsprøver Med Tyre, have been published. The reasons given (10) for initiating these stations were: the usual progeny tests of dairy sires could not be carried out because of the abnormal feeding conditions brought about by World War II, and artificial insemination had become so widespread that an early and reliable test of the transmitting ability of the individual sires for production was imperative. In the testing year, 1945-46, six sires were tested, two at each of three stations. In 1956-57, 94 sires were tested and were distributed among 33 stations. In the 12 testing years, 1945-46 to 1956-57, inclusive, 547 sires have been tested. The special test stations are popular and have developed much breeder interest. Mason (14) investigated these testing stations and concluded that they were genetically sound, that they gave accurate results, but that they were expensive to operate.

The rules by which the test stations are operated are listed in (10) and (11). These rules are followed rather closely; however, there are exceptions. Johansson (8) has pointed out and discussed some of these exceptions. Mason (15) concluded that there is little opportunity to select for testing at the stations only the better heifers by a sire. He also indicated that selection on the dam's production probably had not occurred and would have been relatively ineffective if it had.

Other workers have derived and reported the variances between and within progeny groups for milk and fat yield and fat per cent. Johansson (8), working with data on 174 Red Danish Milkrace progeny groups (reported in Afkomsprøver Med Tyre, II to VIII) found that the variances between progeny groups within stations and years were approximately 14.5, 20.2, and 14.0% of the total variances of milk yield, per cent fat, and butterfat yield, respectively. These multiplied by four yield heritabilities of 0.58, 0.81, and 0.56 for milk yield, fat per cent, and fat yield, respectively. Robertson and Mason (18), working with data on 121 Red Danish Milkrace progeny groups, reported in Afkomsprøver Med Tyre I to VI, obtained heritabilities of 0.75 and 0.71 for milk yield and fat per cent, respectively.

The differences between progeny groups at the test stations are clearly much larger than differences usually found between progeny groups in farmer herds. Johansson (8) has pointed out that progeny groups differ significantly in age at

calving, date of calving, length of service period, and weight. Johansson has further suggested that important errors involved in the Danish progeny tests could arise from differences in the state of nutrition and body development of the heifers at the time of calving. Hofmeyr (6), working with data on 51 progeny groups from the Islands of Zealand and Funen, and published in reports II through VIII, concluded that there was no selection of heifers on the basis of their dam's production, but that there was selection on the basis of the average yield of the herds from which heifers were chosen and on the development and condition of the individual heifers relative to age and stage of pregnancy. Venge (22) has concluded that one of the most serious errors in the Danish testing stations is the variation in days in milk, around the average of 304 days.

It is difficult to accept the above suggestions as the real causes of the surprisingly large differences between sire groups, as most of the above-mentioned factors would tend to increase the variation within sires more than they would the variation between sires. In fact, Johansson (8) has shown that the variance between sire groups is only 14.3, 2.7, 1.4, and 8.5% of the total variance of age at calving, date of calving, service period, and body weight, respectively, whereas that within sire groups accounts for the balance. Venge (22) has shown that the variance between sire groups accounted for none of the variance of days in milk. This would be expected, since all groups average 304 days in milk.

A main question to be asked of the progeny-testing station data is how well station tests predict the results to be expected in farmer herds. Hofmeyr (6), working with butterfat yield, found a correlation of .52 between the station tests and the field test and a regression of .23 of the latter on the former. The records made in farmer herds were expressed as deviations from the contemporary herd average, but the sample involved only 18 sires. In another test, involving 29 Red Danish Milkrace sires, Hofmeyr (6) reported correlations of .66 and .70 between the station tests and first and second field tests and regressions of .40 and .38 of the first and second field tests, respectively, on the station test. There was a correlation of .60 between the first and second field tests and a regression of .54 of the second on the first. The station tests and first and second field tests were based on 18.1, 34.4, and 67.4 daughters per sire, respectively. From these correlations and regressions, Hofmeyr concluded that field tests on 35 daughters provided as reliable information on a sire's breeding value as a station test on 18 daughters. He did not state specifically what his measure of reliability was.

Robertson and Mason (18), working with 62 Red Danish sires, found a correlation of .37 and .65 between field tests and station tests for milk yield and fat tests, respectively. The regressions of the field tests on the station tests were .16 and .46 for milk yield and fat per cent, respectively. The field tests were computed from individual deviations from first-lactation contemporaries. There were approximately 80 daughters per sire for the field tests and 18 for the station tests. Robertson and Mason divided their data on a basis of herd averages into a group of high herds and a group of medium and low herds. Test station results and field tests were slightly more closely correlated for the group of high herds than for the group of medium and low herds. If performance in medium and low herds is

the final assessment of a sire, Robertson and Mason found performance in high herds to be a better indicator than performance at test stations for both milk and fat per cent.

The publications cited above have raised several questions about the value of the special Danish dairy sire progeny-testing stations in determining genetic differences between bulls. The objectives of the present study are to determine the variances between and within sire groups at the test stations and in farmer herds, and to determine whether the expected genetic superiority of future daughters in farmer herds is greater for station tests or tests in farmer herds.

SOURCE OF DATA

The data were taken from the published results of the Danish sire progeny testing stations, Afkomsprøver Med Tyre IV-XI, and from field data provided by the Central Recording Office of the Cattle Breeding Societies in Jutland. A brief description of these data is given in Table 1. The data from the Danish sire progeny-testing stations consisted of the milk and butterfat records of 5,454 daughters of 305 Red Danish Milkrace sires. The number of daughters per sire averaged 17.8, with a range of from 13 to 22. The method of choosing the daughters to go to the stations and the feeding and management of the daughters while at the stations is described by Larsen and Østergaard (11) and by Mason (14).

The data from the Central Recording Office in Jutland consisted of the first complete testing-year records of 3,270 daughters of 110 Red Danish Milkrace sires that had been tested at the special testing stations. Of the 110 sires, 104 had 30 daughters each, three had 28 daughters each, and the other three had 26, 21, and 19 daughters each. In an attempt to insure that the daughters were kept in a medium to high level of management, and that the contemporary herd average was measured well, the daughters were taken from herds with a testing-year herd average of at least 160 kg. of butterfat and at least ten or more cows completing test-year records in the year concerned. Not more than three daughters per sire per herd were included in the data and these were chosen at random. The 3,270 daughters were from 2,176 herds.

TABLE 1

Pertinent characteristics of the test station and field data

		No sires	No daughters	Avg. No. daughters per sire	Mean butterfat production (kg.)	Mean butterfat production (lb.)	Within sire and herd standard deviation (kg.)	Within sire and herd standard deviation (lb.)	No. herds
Test station data	A	305	5,454	17.8	195.3	(430.6)	35.8	(78.9)	144[a]
	B	110	1,954	17.8	196.6	(433.4)			67[a]
Field data		110	3,270	29.7	173.6	(382.7)	31.2	(68.9)	2,176

A — All Red Danish Milkrace progeny at testing stations, for the test years 1948-49 to 1955-56, inclusive.

B — Red Danish Milkrace sires tested at testing stations, in the test years 1945-46 to 1955-56, inclusive, on which field data were obtained from the Central Recording Office in Jutland.

[a] Test station-year groups.

The Danish testing year is from October 1 to September 30 and the records used were generally combinations of first and second lactation records. If a cow calved for the first time between October 1 and December 1, the first lactation record up to September 30 of the following year was used as the record. If a cow calved for the first time between December 1 and September 30, the last part of the first lactation and the first part of the second lactation were used as the test-year record. This was done because the herd averages available were for the test year.

Harris *et al.* (4) and Dickerson (2) showed that calendar-year records are essentially equal to lactation records as measures of a cow's producing ability. Johansson and Hansson (9) concluded that the repeatability of lactation records was only a little higher than that of calendar-year records. They found that there was more variation in the age of the cows concerned when the first calendar-year record was used than when the first lactation record was used. Their records were not adjusted for age but for lactation or calendar-year record number.

ANALYSIS OF DATA AND RESULTS

Data from test stations. The analysis of variance for butterfat production is shown in Table 2. The data on milk production were not analyzed, because Robertson and Mason (18) had previously analyzed comparable data which included a part of the same data used in the present study.

The differences between stations in the same year are significant beyond the 0.01 level of probability, as shown in Table 2. These differences could have been caused by environmental differences between stations, or the genetic differences between bulls tested at different stations could have been larger than those between bulls tested at the same station, or both causes may have operated. The differences between years were also significant at the 0.01 level of probability and, likewise, these differences may have been environmental or genetic or both.

Because the differences between years and between stations within years are significant and of considerable magnitude, it seemed advisable for the purposes of this study to consider only the differences between and within contemporary progeny groups at the same station. The differences between progeny groups within stations and years are significant far beyond the 0.01 level of probability, as shown in Table 2.

The sire component is 229 and the variance among paternal half-sisters is 1,280, as is shown in Table 2. From these components of variance an estimate of

TABLE 2

Analysis of variance of the data on butterfat production for test stations (Group A)

Source	D.F.	M.S.	Components of variance
Years	7	52,086[a]	63
Between progeny groups within years	297	6,877	....
Between stations within years	136	8,608[a]	87
Between progeny groups within stations and years	161	5,395[a]	229
Within progeny groups	5,149	1,280	1,280

[a] Significant at .01 level of probability.

TABLE 3

Analysis of covariance of butterfat production at test stations with age (A) and days in milk (D) as the independent variables

	Unadjusted				Adjusted		
	D.F.	M.S.	σ^2_i	$1-R^2_{F\cdot AD}$	D.F.	M.S.	σ^2_i
Within stations and years	5,310	1,404		0.898	5,308	1,262	
Between progeny groups within stations and years	161	5,395	229		161	5,144	223
Within progeny groups	5,149	1,280	1,280	0.890	5,147	1,140	1,140
Heritability			0.61				0.65

heritability of 0.61 is obtained. This estimate is based on the assumption that the paternal half-sisters have nothing in common except the genes they received from their sire.

Johansson (8) reported that 13.2% of the variation in butterfat yield during the testing period resulted from variation between progeny groups at the same station in the same year. If only the variation between and within progeny groups at the same station in the same year had been considered, 14.5% of the variance would have been between progeny groups, and if this were due only to the genes of the sires, the heritability would have been 0.58. This agrees closely with the estimate 0.61.

The data in Afkomsprøver Med Tyre, IV-XI show some variation in the age at first calving, both between and within progeny groups. The age at calving ranged from 657 to 1,341 days, with a mean of 899 days. Days in milk ranged from 232 to 375 days, with a mean of 304 days. All progeny groups were in milk for an average of 304 days. It seemed advisable to adjust the data for these variables. The first power of each was used in adjusting the butterfat production.

The correlations within progeny groups were .137 between butterfat production (F) and age at calving (A), .287 between butterfat production and days in milk (D), and −.099 between age and days in milk. The negative correlation between A and D contains some automaticity, since the later in the fall a heifer calves the older she will be at calving and the fewer days she will be in production at the progeny-testing stations. The multiple correlation between the dependent variable F and the independent variables A and D was .332 within progeny groups and .319 within station years.

Shown in Table 3 are the mean squares between progeny groups within stations and years and within progeny groups, adjusted for age and for days in milk. The adjustment has decreased both the within- and between-sire components, but it decreased the within-sire component more. With the adjusted components a heritability of 0.65 is obtained, as compared to 0.61 as obtained with the unadjusted components. These estimates are approximately three times as large as most other estimates of the heritability of butterfat production.

Data from farmer herds. The data from the Central Recording Office in Jutland were analyzed for the variance between and within the progeny groups by the 110 sires, as shown in Table 4. The analysis was done on a within A.I. center basis, because bulls at different A.I. centers are used infrequently in the

TABLE 4

The analysis of variance of the field data for milk and butterfat production in kilograms

Source	D.F.	M.S.		Components of variance	
		Milk	Fat	Milk	Fat
Herds within A.I. centers	2,141	1,847,149	3,225	89,386	178
Bulls within A.I. centers	75	682,118	1,214	40,540	71
Herds × bulls. (within A.I. centers)	317	284,832	451	−16,506	−92
Within herds and bulls	702	513,697	970	513,697	970
Heritability				0.29	0.27

same herds and the differences between bulls at different centers would be almost completely confounded with herds. The sample mean squares in Table 4 were equated to their expected mean squares and the resulting components of variance are shown in the last two columns of Table 4. The components of variance for milk yield in Table 4 are similar to those found by Mason and Robertson (16) in high-yielding herds. The herds in the present study averaged 4,079 kg. milk and 173.6 kg. butterfat; the high herds in the study by Mason and Robertson (16) averaged 4,390 kg. of milk. For both milk and fat, the components of variance for bulls and within herds and bulls are comparable in size to those reported by Gaunt and Legates (3).

Assuming that the herd by sire interaction components in Table 4 are zero, one gets 0.27 and 0.29, respectively, for the heritabilities of butterfat and milk production. These estimates are slightly higher than most other estimates (5, 12, 13, 16, 19, 20) of the heritability of milk and butterfat yield. Probably they are too high, because the bulls involved were distributed over a period of 10 yr. and a trend in environment over these years would be partially confounded with sires. The data studied by Mason and Robertson (16) covered a period of 6 yr., and the results from their complete analysis would be subject to a similar trend, but for a shorter period.

Because the records used were not age-corrected, the variation within herds and bulls is probably larger than it would have been if age-corrected records had been used. On the other hand, since the records involved were first calendar-year records, age correction would not greatly change the variance within bulls and herds. In fact, the square root of the mean square within bulls and herds, 970, is 31.1 kg., or 63 lb., which is smaller than comparable components for 305-2×-ME lactation records (5, 12, 13, 19, 20).

It is not likely that the component of variance for sires has been seriously affected in this way, since the age distribution for the different sires would be expected to be approximately the same. The use of calendar-year records should not greatly change the differences between sires. On the other hand, any environmental trend occurring over the 10-yr. period during which the records were collected would be partially confounded with sires and thus cause the sire components to be larger than they should be.

To try to avoid this time trend, the test-year herd average corresponding to the test-year record of each daughter was obtained from the Central Recording

TABLE 5

Analysis of variance of the deviations of the test-year production records from the test-year herd averages

	D.F.	M.S.		Components of variance	
		Milk	Fat	Milk	Fat
Between sires	109	1,482,456[a]	2,497[a]	32,125	53
Within sires	3,160	527,477	916	527,477	916
Heritability	....	.....	....	0.23	0.22

[a] Significant at the 0.01 level of probability.

Office in Jutland. Each daughter's test-year record was then expressed as a deviation from its corresponding test-year herd average. The test-year herd average included the test-year record of the daughter.

Weighting these differences according to the inverse of the variances of the differences was considered as the best means of weighting.[1] Computations showed, however, that when the herd size (N) was ten or more and the number of daughters per herd test-year, per sire (K) three or less, the individual deviations could be given equal weights. Hence, the data were restricted to $N \geq 10$ and $1 \leq K \leq 3$; thus weighting of the individual deviations was dispensed with.

Shown in Table 5 are the analyses of variance for the data from the Central Recording Office, when each daughter's test-year record was expressed as a deviation from the test-year herd average. From the mean squares shown in Table 5 the components between- and within-sire components were derived and give the values 0.23 and 0.22, respectively, for the estimates of heritability of milk and fat. These agree closely with the findings of Mason and Robertson (16), who studied similar Danish data, and with other recent estimates (5, 12, 13, 16, 19, 20).

In expressing the records as deviations from the contemporary herd averages, there is a possibility of including in the within-sire components additional extraneous variation associated with age. If the average ages vary considerably from herd to herd, then the differences between the daughters and contemporary herd averages would be partially confounded with the average ages of the herds. It is not likely that the average age of all the herds for one sire would differ significantly from that for another sire. Thus, if the records were adjusted for age, the heritabilities obtained would probably be higher than those given in the preceding paragraph. On the other hand, they probably would not be as high as the values 0.27 and 0.29 that were obtained from the over-all analysis without using the contemporary herd average. If one assumes that age adjustment would change the between- and within-sire components in Table 5 by the same percentages as those in Table 3 were changed, heritabilities would be 0.25 and 0.24 for milk and fat yield, respectively. It is clear that failure to adjust for age has not seriously biased the estimates of heritability.

Relations between field data and test station data. The correlations between the tests at the progeny test stations and the tests in farmer herds for the 110 sires with tests in both are presented in Table 6. In addition, the correlations between

[1] These were weighted according to the formula $\left(\frac{KN^{3/2}}{N^{3/2}+KN^{1/2}-2K^{3/2}}\right)$.

TABLE 6

The correlations between the various progeny means

	Over-all[a]		Within station years[b]	
	Milk	Fat	Milk	Fat
r_{XY}	0.32	0.26	0.48	0.52
r_{XZ}	0.39	0.35	0.61	0.63
r_{YZ}	0.91	0.84	0.95	0.89
$r_{Y_AY_B}$	0.45	0.43	0.45	0.42

X = tests at test stations.
Y = tests in farmer herds based on deviations from the contemporary herd average.
Z = tests in farmer herds based on daughter averages.
Y_A and Y_B = Samples resulting from a random division of Y with no cow in Y_A that is in Y_B.

[a] Based on 108 d.f.
[b] Based on 42 d.f.

contemporary tests in farmer herds are presented. The number of daughters per sire at the testing stations for the 110 sires averaged 17.8, with a range of from 13 to 22, and the average number of daughters per sire in farmer herds was 29.7. The correlations between test station and field results are, thus, correlations between means based on 17.8 and 29.7 observations, respectively. The correlations between the contemporary field tests are correlations between means based on 14.9 observations each. The means Y_A and Y_B were established by taking a random sample of half of the daughters of each sire and calling the mean of these daughters Y_A and the mean of the remaining daughters Y_B. In the two cases where there was an odd number of daughters, a flip of a coin decided whether the last daughter went in Y_A or Y_B. The means Y_A and Y_B are based on the deviations of the daughter's records from the contemporary herd average.

Table 6 shows that the correlations within station years are higher than the over-all correlations. In general, the correlations between field tests are higher than those between station tests and field tests, but these correlations should not be compared directly, since the progeny means are based on different numbers; also, it should be pointed out that the same animals are included in Y and Z. The extremely close agreement of the correlations between Y_A and Y_B for milk and fat on an over-all and within station year basis supports the contention that the use of the contemporary herd average has successfully removed environmental differences between herds.

The expected correlation between the averages of the progeny groups at the test stations X and those averages from field results as measured by Y and Z can be expressed by the following formula.

$$r_{XY} = \frac{g_x}{2}\sqrt{\frac{N_x}{1+(N_x-1)\,t_x}}\; r_{G_XG_Y}\; \frac{g_y}{2}\sqrt{\frac{N_y}{1+(N_y-1)\,t_y}}$$

To express the correlation between two field tests, the terms with x subscripts should be replaced with analogous terms with y subscripts. In the above

formula, g_x and g_y are the square roots of the heritabilities obtained at the test stations and in farmer herds, respectively, N_x and N_y are the corresponding average numbers of daughters per sire, respectively, and t_x and t_y are the corresponding phenotypic correlations between daughters of a sire. In the formula, $t_x = \frac{g_x^2}{4} + C_x$ and $t_y = \frac{g_y^2}{4} + C_y$, where C_x and C_y are the environmental correlations between daughters records at the test station and in farmer herds, respectively.

Values for g_x^2, g_z^2, g_y^2, t_x, t_z, and t_y are listed in Table 7. All values in Table 7 were obtained from the present data, except those for t_x and g_x^2 for milk. The values for t_x and g_x^2 for milk were obtained by using the within-sire component of variance, 541,696, computed by Robertson and Mason (18), and the appropriate variances of the test station means for sires. For example, the expected variance of the means of sires at the testing stations is $\sigma_x^2 = \sigma_s^2 + \frac{\sigma_w^2}{N}$. Substituting the appropriate figures from the present data, and Robertson and Mason's estimate of σ_W^2, one obtains $137{,}830 = \sigma_S^2 + \frac{541{,}696}{17.95}$, which yields 107,652 for σ_s^2 within stations and years. Using this and Robertson and Mason's estimate of the within-sire variance, the values for t_x and g_x^2 for milk in Table 7 were obtained. Those for the over-all basis were derived in a similar way, but letting $\sigma_x^2 = 199{,}186$. These estimated values of g_x^2 are approximately equal in all cases to the weighted means of the estimates obtained by Johansson (8) and by Robertson and Mason (18).

Given the values of g_x^2, g_z^2, g_y^2, t_x, t_z, and t_y, the expected correlations between tests at the test stations and tests in farmer herds can be computed. Using for g_x^2, g_z^2, g_y^2, t_x, t_z, and t_y the values listed in Table 7 and assuming $r_{G_xG_z} = r_{G_xG_y} = 1$ and that $C_x = C_y = C_z = 0$, the first two rows of Table 8 under Expected 1 are obtained. These results differ widely from the actual; therefore, it was then assumed that $g_x^2 = g_y^2 = g_z^2$ equaled 0.23 for milk and 0.22 for fat, and that t_x, t_z, and t_y equaled their respective values listed in Table 7; consequently, C_x and $C_z \neq 0$ and $C_y = 0$. The results are shown in the first two rows

TABLE 7

Estimates of the correlations between paternal half-sibs and the heritabilities derived from these estimates

		Test station results			Field results			
					Deviations from herd averages		Daughter averages	
		Over-all	Within stations and years		Over-all	Within stations and years	Within A. I. associations	
Milk	t_x	0.24	0.17	t_y	0.06	0.06	t_z	0.07
	g^2_x	0.95	0.66	g^2_y	0.23	0.23	g^2_z	0.29
Fat	t_x	0.22	0.15	t_y	0.06	0.06	t_z	0.07
	g^2_x	0.90	0.61	g^2_y	0.22	0.22	g^2_z	0.27

TABLE 8

The within station and year phenotypic and genetic correlations between field tests and station tests for various assumptions

	Milk			Fat		
	Actual	Expected 1	Expected 2	Actual	Expected 1	Expected 2
r_{XY}	0.48	0.71	0.42	0.52	0.69	0.42
r_{XZ}	0.61	0.82	0.49	0.63	0.87	0.48
$r_{Y_AY_B}$	0.45	0.48	....	0.42	0.46	....
$r_{G_XG_Y}$	....	0.68	1.15	....	0.75	1.24
$r_{G_XG_Z}$	....	0.82	1.23	....	0.87	1.30
$r_{G_{Y_A}G_{Y_B}}$	....	0.94	....	....	0.92	....

of Table 8 under Expected 2. These assumptions are probably nearer the true situation than the first ones, as is evidenced by the closer agreement in all cases between the actual and the expected correlations.

The daughters in the farmer herds by each sire were divided at random into two groups. Each daughter's record was then expressed as a deviation from its corresponding contemporary herd average, and two field tests on each sire were computed. These tests were designated Y_A and Y_B and the actual and expected correlations between these field tests are shown in Row 3 of Table 8. The expected and the actual correlations between Y_A and Y_B agree closely.

The last three lines of Table 8 show the genetic correlations that must be assumed if the actual correlation between tests is to equal the expected correlation. Under Expected 1, one would have to accept rather low genetic correlations between field tests and station tests, for the actual correlation to equal the expected. There is no evidence for such a large interaction between heredity and environment (13, 18, and Table 4 of this study). Under Expected 2, one would have to accept genetic correlations larger than one between station and field tests, for the actual to equal the expected. In the case of the two independent field tests, one would have to accept genetic correlations only slightly less than one for the actual to equal Expected 1.

It can be shown that the regression of a future daughter (Y') in a farmer's herd on the test station average (X) is $\frac{N_X\sigma_{S_X}\sigma_{S_Y}r_{G_XG_Y}}{N_X\sigma^2_{S_X}+\sigma^2_{W_X}}$ and that the regression of (Y') on the average obtained in farmer herds, as measured by the deviations from the contemporary herd average (Y) is $\frac{N_Y\sigma^2_{S_Y}r_{G_YG_Y}}{N_Y\sigma^2_{S_Y}+\sigma^2_{W_Y}}$. By utilizing the genetic correlations shown under Expected 1 in Table 8, and the components of variance shown in Tables 3, 4, and 5, and those derived using Robertson and Mason's data (18), the regressions of Y' on X and Y for various N_X and N_Y can be derived. The regressions of Y' on X and Y' on Y for milk are $\frac{N_X}{2.69N_x+13.55}$ and $\frac{N_Y}{1.06N_y+17.47}$, respectively, and $\frac{N_X}{2.77N_x+15.47}$ and $\frac{N_Y}{1.09N_y+18.72}$, respectively, for fat. In Table 9, the regressions for N_X

TABLE 9

The expected regressions of a future daughter in a farmer's herd (Y') on the progeny average at test stations (X) and on the progeny average in farmer herds (Y) as measured from the contemporary herd average[a]

	Milk				Butterfat			
N_X or N_Y	$b_{Y'X}$	$b_{Y'X}\sigma_X$	$b_{Y'Y}$	$b_{Y'Y}\sigma_Y$	$b_{Y'X}$	$b_{Y'X}\sigma_X$	$b_{Y'Y}$	$b_{Y'Y}\sigma_Y$
5	0.19	86	0.22	81	0.17	3.8	0.21	3.2
10	0.25	101	0.36	105	0.23	4.3	0.34	4.1
15	0.28	106	0.45	117	0.26	4.6	0.43	4.6
20	0.30	110	0.52	126	0.28	4.8	0.49	4.9
30	0.32	114	0.61	136	0.30	4.9	0.58	5.3

$$b_{Y'X} = \frac{N_X \sigma_{S_X} \sigma_{S_Y} r_{G_X G_Y}}{N_X \sigma^2_{S_X} + \sigma^2_{W_X}}$$

$$b_{Y'Y} = \frac{N_Y \sigma^2_{S_Y} r_{G_Y G_Y}}{N_Y \sigma^2_{S_Y} + \sigma^2_{W_Y}}$$

N_X is number of daughters on which test at stations is based.

N_Y is number of daughters on which test in farmer herds is based.

σ_{S_Y} and σ_{S_X} are the sire components of variance resulting from tests in farmer herds and at test stations, respectively.

$\sigma^2_{W_X}$ and $\sigma^2_{W_Y}$ are the within-sire components of variance at test stations and in farmer herds, respectively.

$r_{G_X G_Y}$ and $r_{G_Y G_Y}$ are genetic correlations between tests at stations and tests in farmer herds and successive tests in farmer herds, respectively.

[a] Products of these regressions and the respective standard deviations are also shown.

and $N_Y = 5$, 10, 15, 20, and 30 are shown. The regressions of a future daughter (Y') on a test obtained in farmer herds (Y) are, in general, larger than the regressions of a future daughter (Y') on a test obtained at a special test station (X). When N_X and N_Y are 1, 2, and 3, $b_{Y'X}$ is larger or practically as large as $b_{Y'Y}$, but $b_{Y'Y}$ is clearly larger than $b_{Y'X}$ for values of N_X and N_Y larger than three.

To make a more practical comparison of tests at special stations and tests in farmer herds, one should compare the expected genetic gains under the two methods of evaluating dairy sires. The expected genetic gain is $\hat{G} - G = b_{G1}(z/b)\sigma_1$. The regression b_{G1} will be $b_{Y'Y}$ or $b_{Y'X}$ and is shown in Table 9. The values for z/b can be obtained from the areas and ordinates for the normal curve and those for σ_1 from the appropriate components of variance between and within sires. σ_1 is the standard deviation of the index used as the measure of a sire's breeding value. Since z/b would be constant for the pertinent comparisons, it is sufficient to compute $b_{G1}\sigma_1$ for each situation. One could use the correlations between the various tests and a future daughter to show these relationships, but the products of the regressions and standard deviations more clearly show the importance of the variance of the index used and are probably more easily computed.

In Table 9, the various $b_{G1}\sigma_1$ are shown and, in general, the $b_{G_Y Y}\sigma_Y$ are larger than the $b_{G_Y X}\sigma_X$. For milk, the tests in farmer herds yield a slightly higher expected genetic gain in all cases except where N is 5. For butterfat production, the tests in farmer herds result in 0.83 and 0.94 as much gain, for tests based on five and ten daughters, respectively, are about equal for 15 daughters and give 1.02 and 1.08 times as much progress for tests based on 20 and 30 daughters, respectively.

DISCUSSION OF RESULTS

The sire components obtained from the progeny-testing station data are in all cases surprisingly large, and give estimates of heritability more than twice as large as the estimates obtained from the field data. An explanation of these differences is warranted.

Johansson (8) has shown that the progeny groups at the test stations differ in age at calving, date of calving, length of the service period, and body weight. He has suggested that this might partially explain the high heritabilities of milk and butterfat. Table 2 of Johansson's publication shows that for age at calving, date of calving, service period, and body weight, respectively, the variance within progeny groups is 5.7, 36.0, 63.4, and 9.0 times as large as that between progeny groups at the same station in the same year. Any adjustment of milk and fat yield for these variables would likely decrease the within-sire components of variance much more than the between-sire components and would, thus, increase the heritabilities of milk and fat yield rather than decrease them, as has been suggested. A comparison of the adjusted and unadjusted components of variance and estimates of heritability in Table 3 shows this clearly. Johansson (8) has suggested that differences in the state of nutrition and body development at the time of calving may make an important contribution to the sire component of variance. In his Table 2, 8.5% of the total variance of body weight is accounted for by differences between progeny groups, thus suggesting a heritability of 0.34 for weight at slightly less than 3 yr. of age. If only the variances between and within progeny groups at the same station in the same year are considered, the sire component accounts for 10.0%, thus suggesting a heritability of 0.40. Touchberry (21) reported a heritability of body weight of 0.37 at 3 yr. of age. Blackmore *et al.* (1) reported a heritability of body weight of 0.53 at 2 yr. of age. Mason *et al.* (17) reported heritabilities of 0.41 and 0.37 for body weight at the time heifers were received at the Danish test stations and in the following March, respectively. Comparing the estimate obtained from Johansson's data with the others listed above, gives no evidence for more than normal differences between progeny groups in state of nutrition or body development as measured by weight.

Hofmeyr (6) studied the associations between the average butterfat yield in the herds where the heifers were reared and their weight and butterfat yields at the test stations. The regression of weight on the herd average was linear and significant, whereas the regression of butterfat yield on the herd average had significant linear and curvilinear components. These regressions include differences between stations and years and between the different regions of the islands of Zealand and Funen.

If an analysis of variance of herd levels was computed for Hofmeyr's data, the component of variance within sires would probably be much larger than that between sires within stations and years. An adjustment for herd level would, thus, be more likely to increase heritability than to reduce it.

This leads to the conclusion that the differences between progeny groups at the same test station in the same year are so surprisingly large because of factors affecting the heifers at the test stations.

The factors operating at the test stations to make the differences between progeny groups large could be genetic or environmental or both. It has been suggested that the high level of feeding and management at the test stations enables the genetic differences between sires to be more fully expressed than is the case in farmer herds. If this is true, the interaction between heredity and environment is fairly large and the data in Table 4 do not support this. Furthermore, the study by Legates *et al.* (13), and the data presented in Table 4 of the study by Mason and Robertson (16), provide no evidence for an interaction between heredity and environment. Mason and Robertson (16) have presented evidence that there is an interaction between heredity and environment, in reporting heritabilities of 0.05, 0.12, and 0.22 for low, medium, and high herds, respectively. On the other hand, the high herds studied by Mason and Robertson (16) had a higher average yield of milk than did the comparable groups at the progeny-testing stations, yet the heritability of milk yield in the high herds was only 0.22, whereas that at the test station was 0.75. The above evidence suggests that the increased variation between sires at the test stations, as compared to variation between sires in farmer herds, is environmental in nature.

It has been suggested that some bulls' daughters are more subject to excitability than others and, thus, the progeny averages obtained at test stations are more variable than those obtained from farmer herds. There are no objective data on this contention, but for such a characteristic of a random-mating population one would expect the additive genetic variance within progeny groups to be approximately three times as large as that between progeny groups; thus, differences in excitability alone would not be expected to be the cause of the extremely large heritabilities.

One might conclude that, since the variance within progeny groups at the test stations is as large or larger than that within groups in farmer herds, the test stations have been no more successful in controlling environment than the farmers. It should be pointed out, however, that in random-mating populations the variance within progeny groups contains the environmental variance (σ_E^2) plus three-fourths of the genetic variance ($\frac{3}{4}\ \sigma_G^2$). For example, if one accepts the sire components of variance for butterfat in Tables 3, 4, and 5 as one-fourth of the genetic variance, then the environmental components at the test stations and in farmer herds are 593 and 757, respectively. It is interesting that the estimate of σ_E^2 for farmer herds is 757, whether the actual records or the deviations from the contemporary herd average are used. The sire components of variance obtained from test station data probably contain variation other than $\frac{1}{4}\ \sigma_G^2$; thus, the above estimate of σ_E^2 for the test stations is probably too small. To say that test stations are doing a better or poorer job of controlling environment than farmers, one would have to know σ_G^2 under each system. Using the within-sire standard deviations and the means in Table 1, the coefficients of variation for butterfat production were 18.3, 17.9, and 17.4 for station tests and the two field tests, respectively.

What environmental factors operating at the stations could bring about the increased variation between progeny groups? As noted by Mason (15) and

Robertson and Mason (18), the physical separation of the heifers into distinct progeny groups at the test stations could cause some variation in yield between the groups at the same station in the same year. In addition, at some stations visited it was a practice for one man to milk and partially care for one progeny group while another man milked and partially cared for another. There is keen competition among the test stations to have the high-progeny group and this could cause large differences between station years and between progeny groups at the same station in the same year. The supervisor of the test stations visits each station a number of times during the testing year. During these visits, the persons in charge at the various testing stations are informed as to the progress of the progeny groups at all stations. With this knowledge, and the keen interest in having the high group, it would be natural for the caretakers to push the high group more than the lower groups at a station. Such a practice, if it exists, would inflate the variances between progeny groups at the same station in the same year.

To avoid having environmental factors at a station becoming confounded with progeny groups, it would help to assign a daughter group to more than one station and at random to stalls in the test barns. This could best be done by assigning the numbers to the daughters when they were chosen, and not letting the individual station operators know the parentage of the daughters before the test was completed. It is likely that this would reduce the variation between progeny groups of the same breed at the same station in the same year. It would also seem desirable to withhold official information on the performance of the individual progeny groups from the persons in charge at the various stations until the test year is completed. It is not apparent how providing this information aids in getting an unbiased test; yet, there are a number of ways in which it might encourage biased tests.

The above suggestions have certain faults. They might destroy some of the demonstration value of the station tests and could decrease breeder interest in the tests. It would be impossible to keep the identity of all the animals and the performance of all the groups unknown to the station operators, as they would pick up much of this information from visitors and through their own initiative.

The fact that the correlations under Expected 1 in Table 8 are considerably larger than the actuals, and that the genetic correlations that must be assumed if these expecteds are to equal the actuals are considerably less than one, suggests that heritability at the test stations is less than the estimated values. On the other hand, the fact that under Expected 2 the expected correlations are less than the actuals, and the genetic correlations that must be assumed for the expected to equal the actual are greater than one, suggests that heritability at the stations is higher than it is in farmer herds. If the genetic correlation is set equal to one and the expected correlation is set equal to the actual correlation, r_{xy}, solutions for g_x^2 for milk and fat can be obtained. These values are $g_x^2 = 0.31$ for milk and $g_x^2 = 0.34$ for fat. These estimates for heritability at the test stations are not larger than might be expected, since all daughters calve within a narrow time-interval at the same time of year, all are in their first lactations, and the feeding and management are good and quite uniform.

If one accepts the heritability at the test stations as 0.31 and 0.34 for milk and butterfat yield, respectively, then the genetic correlations must be approximately 1.0, and C_x must be approximately 0.09 and 0.07 for milk and fat, respectively.

The regressions and the expected genetic gains shown in Table 9 afford means of comparing tests in farmer herds with tests at special test stations, for predicting the performance of future daughters in farmer herds. The regressions alone suggest a decided advantage for the tests in farmer herds, but these regressions do not consider adequately the standard deviations of the indices (σ_I) used as criteria for selection. The expected genetic gains shown in Table 9 are, thus, a more valid comparison of tests at test stations with tests in farmer herds. The expected genetic gains suggest a slight advantage for tests in farmer herds and this advantage increases as the number of daughters per sire increases.

The question of how many daughters per sire should be tested can be answered partially by comparing the expected genetic superiority of the sires resulting from various combinations. Suppose that in Denmark 50 tested sires are needed each year and that spaces for 2,000 daughters are available at test stations. Should 100 sires be tested with 20 daughters each, or should 400 sires be tested with five daughters each? In the first situation, one would have to keep the top one-half of the sires to have 50 tested sires; whereas, in the second situation he would keep only the top one-eighth. If one tested 100, 200, 400, or 500 sires per year with 20, ten, five, or four daughters each, for the respective situations, the expected genetic superiority of the 50 sires is 88.1, 127.6, 141.7, and 142.7 kg. for milk and 3.8, 5.5, 6.2, and 6.2 kg. for butterfat. From this, it is quite evident that to make more genetic progress more sires should be tested with fewer daughters each. It should be pointed out, however, that the number of bulls that can be tested reaches a practical limit as testing and holding more bulls entail additional costs.

Perhaps the most serious limitation of dairy sire progeny-test stations is the number of sires that can be tested. This is assuming that the apparent environmental differences confounded with sires can be controlled by proper experimental design and that the expense of operating test stations need not be high. If only a small number of sires can be tested at test stations, then the selection differential will necessarily be so low that little selection can be based on the results. In an area where a high percentage of the herds are tested and artificially bred it seems unnecessary to have special stations. This is especially true if the only purpose of these stations is the testing of the yield of sire progeny groups.

ACKNOWLEDGMENT

We express our appreciation to Mr. Harald Rasmussen, Secretary of the Cattle Breeding Societies in Jutland, for providing the records made in farmer herds.

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UTILIZATION OF VARIOUS SUGARS BY THE YOUNG DAIRY CALF [1,2]

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SUMMARY

Twenty-one fasted male calves, whose ages ranged from 1 to 14 wk., were studied to test their ability to utilize several chemically pure sugars and two commercial corn syrups. The levels of blood-reducing sugars, determined at regular intervals over a period of 4 hr. following the oral administration of the sugars, were used as the measure of utilization of each sugar studied. Sugars in aqueous solution were fed to calves following a 24-hr. fast, at the rate of 2 g. of sugar per pound of body weight. Glucose, lactose, and galactose gave marked elevations in blood-reducing sugars and produced no diarrhea. Calves were unable to utilize sucrose, while the uptake of fructose and maltose was comparatively low. Diarrhea resulted from the feeding of each of these sugars. Sucrose inverted by invertase or citric acid was readily utilized.

In recent years, the high cost of fluid milk, in some areas, has encouraged the use of milk replacers in the diet of young dairy calves. Most milk replacers, however, contain a large proportion of milk products, such as dried skimmilk or dried whey. From the cost standpoint, the use of starch, corn syrup, molasses, distillers dried solubles, and other plant by-products would seem desirable, providing the young calf could utilize these carbohydrates normally.

Numerous studies have shown that the 1- to 8-wk.-old calf is unable to utilize starch (2, 3, 5, 10, 13, 14, 15, 18, 21–24). Larson *et al.* (13, 14) observed that calves could utilize maltose at 9 mo. and later at 5 mo. of age, while Dollar and Porter (2) reported maltose utilization at 9 wk. of age. Recently (2, 9), young calves were reported to have little or no invertase [4] activity and thus were unable to utilize sucrose.

Some investigators (2, 13, 14, 27) agree that glucose may be utilized by young calves, while other workers (4, 6) have reported only a slight utilization of glucose. Flipse *et al.* (5), however, observed a rapid rise in blood sugar levels in 28- to 35-day-old calves fed synthetic diets containing glucose, reaching higher levels than those obtained from lactose, corn syrup, or starch feeding.

Other workers have observed lactose utilization during the first 2 or 3 wk. in the life of the calf, with a lowered utilization becoming evident as the calf becomes older (2, 8, 9). This was believed to be due to a decline in intestinal lactase activity. When limited amounts of lactose were fed over longer periods, the growth was normal (4, 5, 6, 20, 25); whereas, doubling the lactose intake (19, 20) lowered utilization and produced diarrhea.

The present study was initiated to further investigate the degree to which certain sugars could be utilized, when orally administered at regular intervals to fasted calves of varying ages.

Received for publication July 20, 1959.

[1] Taken from data submitted to the graduate faculty of the University of Illinois by the senior author in partial fulfillment of the requirements for the M.S. degree.

[2] This project was supported in part by the Illinois Farm Supply Company, Chicago.

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[4] Invertase used in this study was obtained from Nutritional Biochemicals.

EXPERIMENTAL PROCEDURE

Management of calves. Twenty-one male calves, reared for the first two days on colostrum, were randomly assigned on the third day of life to one of the carbohydrate groups under study. Calves were housed in individual pens which were bedded with crushed corncobs. Whole milk was fed twice daily, with calf starter, good-quality alfalfa hay, and trace-mineralized salt. Clean water was available throughout the experimental period.

Carbohydrate feeding. At approximately seven-day intervals, the calves were fasted for a period of 24 hr., after which they received, by means of nipple pails, warm water solutions of one of the following sugars: lactose, galactose, glucose, sucrose, fructose, maltose, sucrose inverted by invertase, sucrose inverted by citric acid, sucrose plus invertase, sucrose plus 25 g. brewers' dried yeast, corn syrup (Staley), or white corn syrup (Karo). These carbohydrates were fed at the rate of 2 g. per pound of body weight in a minimum of water. With the exception of the commercial corn syrups, all sugars used were chemically pure. Invertase additions are indicated in Table 1 footnotes.

Invert sugar preparation. A 60% sucrose solution was acidified to a pH of 4.5 by adding 0.1 *N* HCl. To this solution, 1.55 parts by weight of invertase were thoroughly mixed. The mixture was stored at a constant temperature of 55° C. for a period of 24 hr. prior to feeding. Inversion was approximately 90%.

Method of inverting sucrose by citric acid. To a solution of 70 parts sucrose and 30 parts of distilled water, 1.3 oz. of citric acid were added. The mixture which was heated, with stirring, to 97° C. and held for 0.5 hr., had a pH of 4.5.

Blood samples. Jugular blood samples (heparinized) were taken before feeding, and at 0.5, 1.5, 3.0, and 4.0 hr. after feeding.

Blood analysis. Blood samples were analyzed for reducing sugars, using the method of Nelson-Somogyi (17).

DISCUSSION OF RESULTS

The results of the studies are summarized in Table 1.

The blood sugar levels of the fasted calves stabilized at the 3rd wk. A number of workers (1, 11, 12, 16, 26) agree that blood sugar levels of calves fed normal rations decrease from a range of 80 to 113 mg.% during the first week of life, to a range of 40 to 75 mg.% by the time the calf is 6 wk. of age.

One hour after administering lactose to three calves 1 wk. of age, the blood sugar level rose from 107 to 203 mg. % and remained high throughout the 4-hr. period. At 2, 3, and 4 wk. of age, the level of blood sugar following sugar administration did not reach as high a level as at 1 wk., but from 5 to 8 wk. the levels were again nearly as high as at 1 wk. of age. This agrees with the work of Flipse *et al.* (4, 5, 6), but not completely with that of Dollar and Porter (2), whose work indicated lactose utilization up to 15 days of age with a lowered utilization at 30 to 34 days of age. No scours were observed following the oral administration of lactose.

The feeding of galactose produced somewhat the same pattern as lactose for the first 4 wk. tested. Galactose absorption produced approximately the same

TABLE 1

Average total blood reducing sugars prior to and following intake of various sugars in calves fasted for 24 hr.[a]

Time[f]	Lactose	Galactose	Glucose	Sucrose	Fructose	Maltose	Sucrose inverted by Invertase	Sucrose inverted by Citric Acid	Sucrose + Invertase	Corn syrup Staley	Corn syrup Karo	Sucrose + brewers yeast
	Average level of total blood reducing sugars *(mg/100 ml. blood)*											
(hr.)	1-wk.-old calves											
	(3)[c]	(2)	(4)	(3)	(1)				(1)[d]			
Before[b]	107	87	103	75	83				114			
+1	203	298	221	78	129				342			
+2	281	271	225	77*	120				580			
+4	223	236	138	72	96				200			
	2-wk.-old calves											
	(4)	(2)	(4)	(4)		(4)			(2)[e]			
Before	67	64	77	71		82			80			
+1	144	176	224	71		87			186			
+2	170	172	218	76*		87			251			
+4	168	149	180	72		81*			214			
	3-wk.-old calves											
	(4)	(2)	(4)	(4)		(4)			(2)[g]			
Before	61	71	68	62		60			50			
+1	168	173	272	75		92			200			
+2	191	215	217	74*		95			279			
+4	147	172	201	65		82*			272			
	4-wk.-old calves											
	(4)	(2)	(4)	(4)								
Before	57	77	62	63								
+1	186	194	261	68								
+2	189	186	286	72*								
+4	156	229	229	67								
	5-wk.-old calves											
	(3)		(4)	(5)					(5)[g]		(4)	
Before	65		60	62					61		63	
+1	195		282	70					179		103	
+2	233		361	68*					200		104	
+4	205		275	64					243		81*	

TABLE 1 (Concluded)

Time †	Lactose	Galactose	Glucose	Sucrose	Fructose	Maltose	Sucrose inverted by Invertase	Sucrose inverted by Citric Acid	Sucrose + Invertase	Corn syrup Staley	Corn syrup Karo	Sucrose + brewers yeast
	Average level of total blood reducing sugars *(mg/100 ml. blood)*											
(hr.)	6-wk.-old calves											
	(4)		(3)	(5)			(3)	(2)				
Before	61		55	60			59	52				
+1	184		262	64			174	116				
+2	261		385	64*			206	157				
+4	204		443	65			162	114*				
	7-wk.-old calves											
	(2)		(2)	(4)	(3)				(2)[g]			
Before	66		56	62	68				75			
+1	193		186	70	104				214			
+2	222		314	68*	99*				243			
+4	222		300	62	90				189*			
	8- to 10-wk.-old calves											
	(3)		(3)	(12)			(6)			(4)		(3)
Before	61		56	61			64			60		64
+1	209		149	67			204			169		79
+2	233		262	69*			267			196		78*
+4	201		276	63			162			115*		70
	11- and 12-wk.-old calves											
				(9)			(2)	(4)	(3)[h]			
Before				65			64	57	84			
+1				72			186	191	295			
+2				72*			187	243	219			
+4				69			149	167	106*			
	13- and 14-wk.-old calves											
				(4)			(5)			(1)[h]		
Before				62			67			80		
+1				69			191			243		
+2				72*			208			243		
+4				69			138			69*		

* Scoured.
† Time related to sugar feeding.
[g] Sugars fed at 2 g/lb body weight.
[h] Blood-reducing sugar levels before the feeding of sugars.
[c] Figures in parentheses indicate number of calves.
[d] 20 ml. of invertase added to sugar solution at feeding time; [e] 10 to 20 ml.; [f] 10 ml.; [g] 2.5 to 5.0 ml.; [h] 4 to 5 ml.

rise in blood reducing sugar level as was observed in lactose feeding. Again, the blood sugar reached a lower maximum level at 2 wk. of age than at 1 wk., as was observed for lactose. No scours resulted when galactose was fed.

Glucose feeding gave an excellent blood reducing sugar response of 221 mg.% 0.5 hr. after feeding. The level remained high up to the 3rd hr., after which there was a decline. At the ages of 2 and 3 wk., the maximum blood sugar level nearly equalled that of the 1st wk., as opposed to the results observed when lactose and galactose were fed at these ages. At 4 to 7 wk. of age, the blood sugar level rose rapidly at 0.5 to 2.0 hr. and remained fairly high up to the 4th hr. following glucose feeding, while at 6 wk. of age, the average blood sugar level rose rapidly and remained high through the 4th hr. after feeding. Of all the sugars tested, the highest rise in blood sugar followed glucose administration, confirming the results of Wiese *et al.* (27). These results are not in agreement, however, with the results of Flipse *et al.* (4, 5, 6) when glucose was fed. There was no evidence of scours observed after glucose feeding.

Only slight fructose utilization was observed throughout the 8-wk. period of feeding. All calves given fructose scoured severely. These results do not necessarily conflict with the work of Goetsch *et al.* (7), since fructose was injected intravenously in their studies.

Little or no maltose utilization was observed at the 2nd and 3rd wk. This was in contrast to the work of Dollar and Porter (2), who found appreciable utilization of maltose in calves 9 wk. of age. Larson *et al.* (13, 14) reported maltose utilization at 5 and 9 mo. of age. At 2 wk. of age, the average blood sugar level rose from 82 to 87 mg.%. Calves scoured severely 3 to 4 hr. after maltose feeding. At 3 to 4 wk. of age, the average blood sugar level rose from 60 to 99 mg.%, with scouring continuing for 5 hr. after feeding.

Calves were unable to absorb sucrose throughout the experimental period of 13 wk. This agrees with other reported work in which sucrose was fed to calves (2, 9). Within 0.5 to 2 hr. after sucrose feeding, the calves scoured severely. The average blood sugar level did not rise more than 10 to 13 mg.% over the fasted sugar level at any time throughout the 13-wk. study of sucrose.

Invert sugar, produced by adding invertase to the sucrose solution, was utilized well by calves at 6 to 8 wk. of age. Within 1 hr. after feeding the invert sugar, the average blood sugar level rose from 59 to 174 mg.% and reached a maximum of 206 mg.% at the 2nd hr. It remained high at the 3rd hr. and then dropped at the 4th hr. From 9 to 15 wk. of age, the rise in the blood sugar level was a little higher than at the 6th and 8th wk. No scouring resulted from invert sugar feeding when the pH of the sugar solution was elevated, following the inversion process, to 7.0 by the use of $NaHCO_3$; however, when the pH was lowered to 4.5 and 5.0, scouring occurred in most cases within 4 hr.

Invert sugar, inverted by citric acid, was well utilized at 6 wk. of age. After the feeding of the invert sugar to 6-wk.-old calves, the average blood sugar level rose within 2 hr. from 52 to 157 mg.% and declined to 114 mg.% after 4 hr. The blood sugar levels of 12- and 13-wk.-old calves rose from 57 mg.% to a maximum of 243 mg.% at the 2nd hr. after feeding. The pH of the invert sugar was

between 4.0 and 4.5. Scouring resulted in most of the calves around the 3rd to the 4th hr. after feeding citric acid inverted sugar. No neutralization of this particular sugar solution was tested.

When 20 ml. of invertase was added to a sucrose solution and fed immediately to a 1-wk.-old-calf, the rise in blood sugar was from 114 to 271 mg.% in 0.5 hr.; and to 580 mg.% at the 2nd hr., after which it fell to 200 mg.% at the 4th hr. At 2 and 3 wk. of age, the average blood sugar level of two calves rose to a maximum of 329 mg.% in 3 hr. The sucrose solution contained 10 to 20 ml. of invertase, depending on the amount of sugar fed. At the 5th wk., when only 2.5 to 5.0 ml. of invertase were added to the sugar solution, the average maximum sugar level in the blood of five calves reached 243 mg.% at the 4th hr. No scours resulted in these calves. At 7, 11, and 13 wk. of age, when the calves were fed the sucrose solution to which 2.5–5.0 ml. of invertase was added, the average blood sugar level rose from 75 to 243 mg.% at 1.5 hr., with calves scouring frequently from the 3rd to the 4th hr.

The addition of 25 g. of dried brewers' yeast, as an invertase source to sucrose, gave very little response. At 7 to 10 wk. of age, following the feeding of sucrose plus brewers' yeast, the average blood sugar level rose from 64 mg.% to a maximum of 81 mg.% at 1.5 hr. and at the 4th hr. it declined to 70 mg.%. The calves scoured severely within 2 hr. after feeding the sucrose with brewers' yeast.

The corn syrup (Staley) was only fairly well utilized. After feeding corn syrup to four calves, aged 8 to 11 wk., the average blood sugar level rose from 60 to 106 mg.% at 0.5 hr., reaching a maximum of 205 mg.% at 1.5 hr. after feeding. At the 4th hr., it dropped to 115 mg.%. Calves scoured mildly at 4 hr. after feeding this corn syrup.

The feeding of corn syrup (Karo) produced very little rise in blood sugar. After feeding corn syrup (Karo) to four calves at 5 wk. of age, the average blood sugar level rose from 63 mg.% to a maximum of 104 mg.% at the 2nd hr. At the 4th hr., the sugar level declined to 81 mg.%. The calves scoured frequently from the 4th hr. after the feeding of corn syrup (Karo).

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STUDIES ON 9α-FLUOROPREDNISOLONE ACETATE, A NEW SYNTHETIC CORTICOSTEROID FOR THE TREATMENT OF BOVINE KETOSIS

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SUMMARY

A new synthetic corticosteroid, 9α-fluoroprednisolone acetate, Predef ®[1], has been shown to be approximately ten times as potent as prednisolone in its ability to elevate blood glucose in normal lactating dairy cows. Blood mineral studies demonstrated the influence of 9α-fluoroprednisolone acetate on phosphorus, sodium, potassium, calcium, and magnesium. 9α-Fluoroprednisolone acetate is indicated in the treatment of bovine ketosis.

Since the successful treatment of bovine ketosis with cortisone and hydrocortisone in 1950 (7), a number of synthetic glucocorticoids have been recommended for this use (13), and research continues to develop more potent steroids to do more specific jobs. The purpose of this paper is to suggest 9α-fluoroprednisolone acetate for the treatment of bovine ketosis.

The biological activity of 9α-fluoroprednisolone acetate was first reported in 1955 by Stafford *et al.* (16). Their work in the rat demonstrated that this compound was about 50 times as potent as hydrocortisone as a gluconeogenic agent (measured by the liver glycogen deposition assay), and almost 20 times as potent as desoxycorticosterone acetate in causing sodium retention in the adrenalectomized rat. Dulin (3) reported 9α-fluoroprednisolone to be 14 times as potent as hydrocortisone as an anti-inflammatory steroid as measured by the cotton pellet implantation assay. Data such as these prompted experiments leading to the possible use of this compound in the treatment of bovine ketosis.

Shaw (12) indicated that 9α-fluoroprednisolone was approximately twice as potent as 9α-fluorohydrocortisone and that the recommended dose of 9α-fluoroprednisolone was 25–50 mg. Goetsch, McDonald, and Odell (6) reported 9α-fluoroprednisolone to be about four times as potent as prednisolone or 9α-fluorohydrocortisone in its ability to elevate blood glucose of normal dairy cows. The following experiments elucidate further some of the blood chemistry responses obtained in normal dairy cows with this interesting steroid at a variety of dose levels.

EXPERIMENTAL MATERIALS AND METHODS

Normal lactating Guernsey cows were placed in stanchions in the Upjohn Veterinary Experiment Station dairy barn two or three days before the experiment started, to allow the animals to become acclimated to these quarters and to dry feed. During the day the cows were allowed to exercise in a dry lot. Sterile aqueous suspensions of the steroid were administered intramuscularly in the cervical area according to the schedule in Table 1. 9α-Fluoroprednisolone (9FΔF) and its 21-acetate (9FΔFAc) were prepared at a concentration of 1 mg. steroid

Received for publication December 21, 1959.

[1] The Upjohn trademark for 9α-fluoroprednisolone acetate is Predef.

TABLE 1

Treatment schedule for the various experiments

Experiment No.	Group No.	No. cows	Treatment	Determinations
I	1	9	One 10 mg. 9FΔF dose	Glucose
II	1	5	One 10 mg. 9FΔFAc dose	Glucose, phosphorus, milk production
	2	2	One 20 mg. 9FΔFAc dose	
	3	7	One 100 mg. ΔF dose	
	4	2	One 200 mg. ΔF dose	
III	1	2	10 mg. 9FΔFAc daily for 7 days	Glucose, phosphorus, sodium, potassium, calcium, magnesium, and milk production
	2	2	7.5 mg. 9FΔFAc daily for7 days	
	3	2	5.0 mg. 9FΔFAc daily for 7 days	
IV	1	2	100 mg. 9FΔFAc daily for 2 days	Glucose, phosphorus, sodium, potassium, calcium, magnesium, and milk production
	2	2	50 mg. 9FΔFAc daily for 2 days	
V	1	2	Sodium propionate ¼ lb. twice a day orally for 6½ days; 10 mg. 9FΔFAc daily for 2 days on the 4th and 5th morning of NaP treatment	Glucose, phosphorus, sodium, potassium, calcium, magnesium, and milk production
	2	2	Sodium propionate ¼ lb. twice a day orally for 6½ days; 20 mg. 9FΔFAc daily for 2 days given on the 4th and 5th morning of NaP treatment	

per cubic centimeter of vehicle. The prednisolone (ΔF) concentration was 10 mg/cc.

One oxalated and one clotted blood sample for the preparation of serum were collected each time a sampling was made. Blood samples were collected at arbitrary intervals before and after treatment for the determination of glucose (15) and serum sodium (8), potassium (8), inorganic phosphorus (4), calcium (17), and magnesium (17). Milk production was recorded and any changes in the normal physical condition of the animals were noted. Except for Experiment I, the results were expressed in per cent of the pretreatment levels observed for each group, to permit easier evaluation of the results.

RESULTS

Experiment I. In comparing the glucose effect of ΔF and FΔF that had been determined in rats (i.e., 9α-fluoro-compound was approximately ten times as potent as the parent compound), and extrapolating this information from the recommended dose of prednisolone in cows (*ca.* 100 mg.), one would arrive at a dose of 10 mg. 9FΔF for cows. Upon administering a 10-mg. dose intramuscularly to nine cows (Figure 1), the blood glucose began to rise significantly above

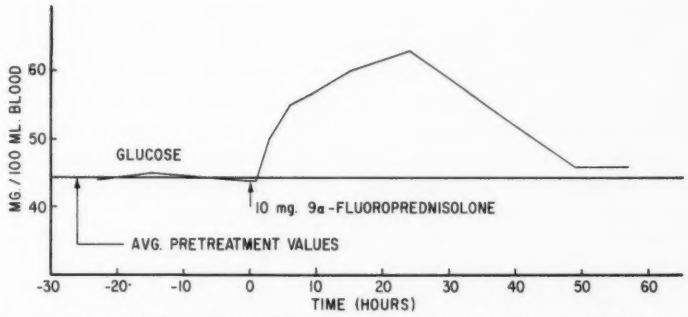

FIG. 1. Effect of 10 mg. FΔF I.M. on the blood glucose of nine normal dairy cows.

pretreatment values about 3 to 6 hr. later and reached peak levels approximately 24 hr. after the steroid was given. The elevated values persisted for about 48 hr. before returning to pretreatment levels.

Experiment II. 9α-Fluoroprednisolone was available in two forms, the free 21-alcohol and the 21-acetate—the latter was used in all subsequent experiments. In confirming the initial experiment, a direct comparison of prednisolone (100- and 200-mg. doses) with 9FΔFAc (10 and 20 mg.) is shown in Figure 2. The

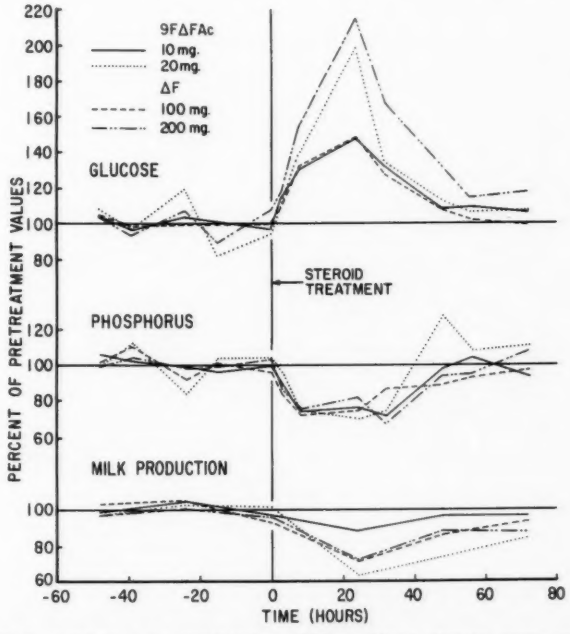

FIG. 2. Effect of FΔFAc and Prednisolone I.M. on blood glucose, serum phosphorus, and milk production in normal dairy cows.

blood glucose of the cows receiving 10 mg. 9FΔFAc (Group 1) or 100 mg. ΔF (Group 3) rose to 150% of pretreatment levels 24 hr. after administration of the steroid and returned to normal levels in 48 hr. The cows receiving 20 mg. 9FΔFAc (Group 2) or 200 mg. ΔF (Group 4) produced a 100% increase in blood glucose in 24 hr. and returned to normal values in 56 hr. These results paralleled the rat data, in that 9FΔFAc was about ten times as potent as ΔF in its ability to elevate blood glucose. The duration of action of the two steroids was approximately the same when compared at equivalent dose levels.

Both steroids produced a transitory drop in serum phosphorus which lasted as long as the blood glucose was elevated.

Milk production was depressed by each steroid during the therapy period. Both dose levels of prednisolone and the high level of 9FΔFAc produced a 30–35% drop in milk output for the 24-hr. period following treatment. Milk flow began to return to normal during the next two days. The 10-mg. dose 9FΔFAc produced only a 10% decrease in milk output during the experimental period.

Experiment III. The influence of repeated administration of 9FΔFAc was studied by treating cows daily for seven days at three different levels, 10, 7.5, and 5.0 mg/cow/day (Figure 3). For each dose level a definite increase in blood

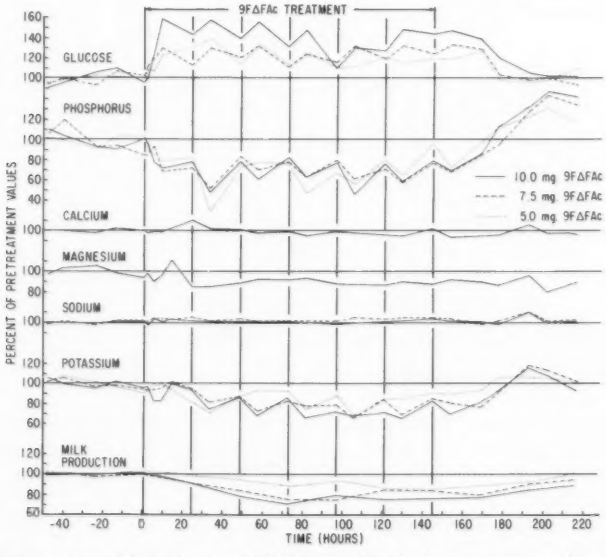

FIG. 3. Effect of multiple doses of FΔFAc on blood glucose, serum minerals, and milk production of normal dairy cows.

glucose was observed 9 hr. after the steroid was administered, which was followed by a drop to lower levels 24 hr. after injection. The lower blood glucose levels noted 24 hr. after injection were still, in each case, substantially above pretreatment levels. On repeated doses the cows continued to respond with significant

increase in blood glucose. The response produced by the 10-mg. dose was significantly higher than that produced by the 7.5- or 5.0-mg. level; however, there was no significant difference between the two lower dose levels.

Each time an injection was made, serum phosphorus dropped to 60–70% of treatment values and then rose to about 80% of pretreatment levels 24 hr. later. As soon as treatment was terminated, the values rebounded to levels as much as 40% above pretreatment levels before returning to normal values.

No significant changes were observed in serum calcium (10.3 mg. %), magnesium (4.0 mg. %), or sodium (144 meq/liter) of the cows receiving 10 mg. 9FΔFAc.

Potassium values changed very little after the initial injection, but dropped following the second treatment to 70–80% of pretreatment level (4.35 meq/liter). Serum potassium remained low throughout the experiment period and returned to normal values about 48 hr. after the last treatment. During this period of hypokaliemia, no untoward clinical signs were evident in the animals.

Milk production decreased 25–30% at the 10.0- and 7.5-mg. levels during the treatment period, but only a slight depression occurred at the 5.0-mg. level. Production returned to 90% of pretreatment levels 4–5 days after the last injection.

Experiment IV. In an attempt to determine clinical levels of safety, two intramuscular injections 24 hr. apart of 50 mg. 9FΔFAc were made in each of two cows. Also, two doses of 100 mg. 9FΔFAc were given intramuscularly to each of two additional cows.

The blood glucose values rose 70–80% above pretreatment and persisted seven days at 15–20% above normal values (Figure 4). Serum phosphorus dropped

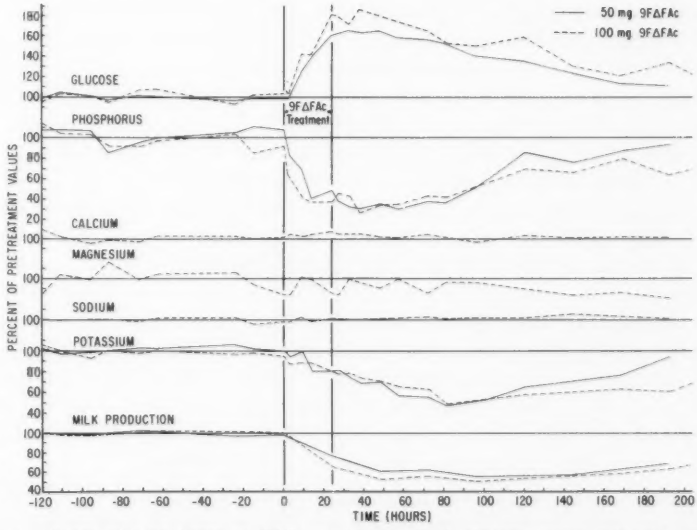

FIG. 4. Effect of high doses of FΔFAc I.M. on blood glucose, serum minerals, and milk production of normal dairy cows.

to 30–40% of pretreatment values and remained at this low level 3 to 4 days before returning slowly to pretreatment levels 6 to 7 days later. The animals appeared clinically normal throughout the experiment. Serum calcium, magnesium, and sodium were not significantly altered from pretreatment values.

The potassium levels in the serum decreased gradually, reaching a level some 50% of pretreatment values about 3¼ days after the initial injection. The two cows receiving 50 mg. 9FΔFAc showed a gradual return to normal levels, being complete eight days after the steroid was first administered. The cows receiving 100 mg. 9FΔFAc remained at reduced levels 60-70% of pretreatment levels throughout the experimental period. A check of the serum nine days later indicated the potassium had returned to pretreatment values. No clinical signs of hypokaliemia were noted during the experiment.

The milk production of the cows in both groups dropped to 40–50% of pretreatment level. The recovery toward normal production was quite slow.

Experiment V. The use of sodium propionate (NaP) orally for the prevention or treatment of bovine ketosis has been reported by Schultz (10, 11). The report by Shaw *et al.* (1, 14) of the adverse effects (hypokaliemia) of potent fluoroglucocorticoids (fluorohydrocortisone) on ketotic cattle and on animals given additional sources of sodium led to the following experiment. One-fourth pound of sodium propionate was administered to four cows morning and evening by drenching for 6½ days. Drenching with sodium propionate was necessary because previous tests revealed that the cows would not eat it when admixed with the feed. On the fourth and fifth day of drenching, 10 mg. 9FΔFAc was given intramuscularly to one group and 20 mg. of the steroid to the second group.

The three-day oral treatment of sodium propionate prior to the administration of the steroid produced no significant change in blood glucose (Figure 5). However, after steroid administration, glucose values rose to expected levels of 50–70% above normal, with the 20-mg. dose producing the greater response for a longer time. The increase in blood glucose following the 10-mg. dose with sodium propionate was not significantly different from that observed in previous experiments in which only a 10-mg. dose of steroid was given.

During the initial phase of the experiment, sodium propionate alone did not affect serum phosphorus; however, as soon as the steroid was given, serum phosphorus of both groups dropped sharply, reaching values 45–50% of pretreatment levels shortly after the second injection. These values were lower than those reported in the other experiments and may indicate some influence by the sodium propionate. The serum phosphorus of the sodium propionate–treated cows which received 10 mg. 9FΔFAc returned to normal in about three days after the last injection. However, the serum phosphorus in the animals receiving 20 mg. 9FΔFAc rose to 50% above pretreatment values within four days after the last injection. Thereafter, there was a gradual return to normal.

Serum calcium, magnesium, and sodium were not significantly altered from pretreatment levels at the 20-mg. dose level. These constituents were not measured at the 10-mg. dose level.

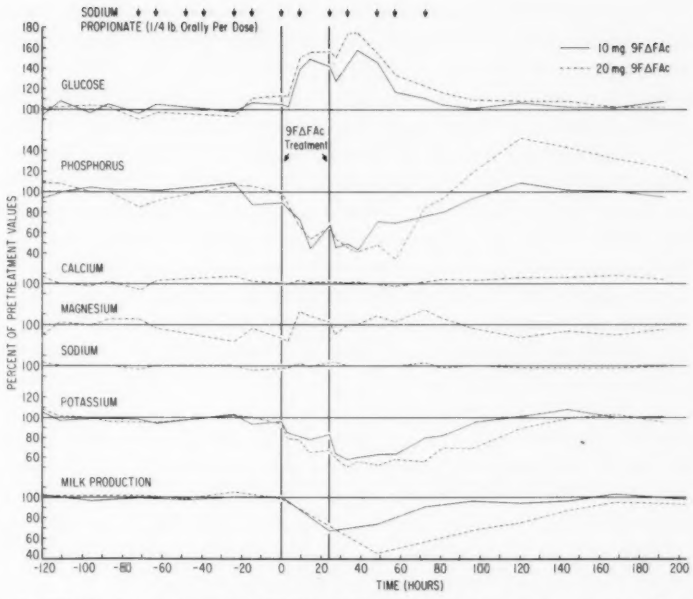

FIG. 5. Effect of superimposing FΔFAc I.M. on blood glucose, serum minerals, and milk production of normal dairy cows receiving sodium propionate orally.

Serum potassium was significantly lowered in both groups 3 hr. after the steroid injection and continued to fall, reaching 50–60% of pretreatment values about 9 hr. after the second injection and remained at this level for 30–40 hr. The recovery of serum potassium to normal levels required 40–50 hr. in those cows receiving sodium propionate; whereas, in the earlier experiments, only 15–20 hr. were required.

Nine hours after the second injection, at the time serum potassium fell to its its lower levels, both cows on the 20-mg. dose level exhibited a slight but definite depressed attitude, as evidenced by slower consumption of grain, a drop in hay consumption, and loose stools. Both cows were brighter, alert, and back on full feed within 24 hr. after the onset of symptoms. The cows on the 10-mg. dose remained normal in appearance.

Milk production, though not affected by sodium propionate administration, was reduced markedly according to the amount of steroid given. The 10-mg. dose caused a 35% drop, with complete recovery in four days after steroid treatment began; whereas, the 20-mg. dose caused a 55% drop, with a 95% recovery in seven days.

DISCUSSION

These studies showed that 9FΔFAc, one of the many highly potent analogues of the natural occurring adrenal cortical steroids, was ten times as effective as prednisolone in raising the blood glucose level of normal dairy cows. The tenfold

increase in potency of the fluoro-steroid over ΔF was somewhat higher than that reported by Goetsch *et al.* (6). This difference may be attributed to variations in experimental conditions.

A possible explanation for the increased potency of the 9FΔF over ΔF is the difference in the rates of reduction of Δ^4-3 and C_{20} ketone groups of ΔF and 9FΔF by a liver enzyme system. It has been shown that the ketone groups of prednisolone were reduced to biologically inactive products more than twice as rapidly as those of the 9FΔF (5). Consequently, 9FΔF remained present in a biologically active form more than twice as long as ΔF. This factor would increase the length of time the active steroid remained in the circulatory system and thus contribute to a longer elevated blood glucose level. Collins (2) demonstrated prednisolone to have a half-life in the blood stream 1.37 times longer than hydrocortisone. Although it has not been reported, the half-life of 9FΔF could be expected to be longer than ΔF, since the fluoro-steroid was metabolized more slowly, as shown by Glenn's work (5).

Cows with primary ketosis occasionally require more than one injection of steroid to bring about recovery (12). Since the cows used in our experiments did not become refractory to repeated doses of 9FΔFAc, it would appear reasonable to expect ketotic cows to respond equally well. Also, the possibility of using 9FΔFAc repeatedly at this low dose, in a prophylactic manner in susceptible cows during the critical period, should be considered in future studies.

The parenteral use of large therapeutic doses of 9FΔFAc (50–100 mg.) appeared to be safe. Even though blood potassium was lowered for a number of days, no clinical signs of hypokaliemia occurred. Of interest was the observation that blood glucose remained elevated for 6 to 7 days when large doses were administered. This indicated that 9FΔFAc was removed from the site of injection and inactivated slowly. Thus, in cases where long-acting therapy is indicated, it would appear that higher doses could be recommended safely.

The reduced inorganic serum phosphorus, as seen in these experiments, and which accompanies glucocorticoid therapy, has been recognized by others (9). It was similar to that observed during glucose loading experiments. The loss of the phosphorus from the serum was probably associated with glucose metabolism; consequently, it did not interfere with calcium metabolism, since no change in serum calcium was noted in these experiments. Because of its transient nature, clinical signs of phosphorus deficiency were not noted in the animals during glucocorticoid administration.

Sodium and magnesium, like calcium, did not vary significantly from normal values during the experimental periods.

Goetsch and McDonald (6) reported no decrease in serum potassium following the administration of a single dose of 100 mg. 9α-fluoroprednisolone alcohol. In our experiments, a definite decrease in serum potassium values was produced by the administration of two doses of 100 mg. 9α-fluoroprednisolone acetate. However, the hypokaliemia was temporary and the serum potassium values returned to normal values shortly after cessation of treatment. Further study is needed to clarify the role of mineralocorticoids in cattle.

The regime of oral sodium propionate apparently contributed to the clinical signs of potassium deficiency observed in the cows receiving 20 mg. 9FΔFAc, as seen in Experiment V, thus confirming the observations reported by Shaw *et al.* (1, 14). This conclusion was further substantiated by noting that the cows in Experiment IV which received no sodium propionate, but did receive much larger doses of 9FΔFAc (50 and 100 mg.), elicited no clinical signs of potassium deficiency. Consequently, caution should be exercised if the two therapies are used concurrently.

Milk production was not seriously reduced, except at the high or repeated dosages of 9FΔFAc. Since ketotic cows are usually in a state of reduced milk output, further decrease in production probably may not occur, or be noticed.

9FΔFAc has been used with gratifying results in the treatment of clinical cases of primary ketosis.[2] These results will be the subject of a future paper.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to A. M. Thornton and R. E. Gosline for technical assistance. The authors gratefully acknowledge the supply of 9α-fluoroprednisolone and 9α-fluoroprednisolone acetate furnished by the Chemistry Department of this Company.

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TECHNICAL NOTES

AN IMPROVEMENT IN THE DIRECT NESSLERIZATION METHOD FOR THE DETERMINATION OF TOTAL NITROGEN IN STORED STERILE MILK

Recently, Murthy and Herreid (4) in their studies on stored milk indicated that the direct Nesslerization method of Hetrick and Whitney (2), though useful in the case of fresh milk, can not be used for the study of nitrogen distribution in stored sterilized milk, because of the variable digestibility of the stored samples. To find a suitable method for the determination of micro-Kjeldahl nitrogen comparable to the macro-method, the following direct Nesslerization method based on those of Lang (3) and Hetrick and Whitney (2) was investigated and found satisfactory.

MICRO-ANALYTICAL PROCEDURE

Reagents

(a) Digestion mixture: 40 g. of K_2SO_4 (C.P.) and 2 ml. of $SeOCl_2$ (technical grade) are made up to 250 ml. with ammonia-free water and this solution is mixed with 250 ml. of H_2SO_4 (C.P.)

(b) Nessler's Reagent: 50 g. of KI (C.P.) is dissolved in 35 ml. of cold ammonia-free water and a saturated solution of $HgCl_2$ (C.P.) is added until the orange precipitate formed persists. 400 ml. of an approximately 9 *N* NaOH solution [300 g. of NaOH (C.P.) in one liter of ammonia-free water] are then added. The solution is allowed to stand for several days before use, for equilibrium to be attained.

(c) Standard ammonium chloride stock solution (1 ml. = 1 mg. N) : 1.910 ± 0.001 g. of NH_4Cl are dissolved in ammonia-free water and diluted to volume in a 500-ml. volumetric flask. Before using, this solution is further diluted with ammonia-free water so that 1 ml. = 0.02 mg. N.

(d) $K_2Cr_2O_7$ solution: The concentration of this solution is determined by the per cent transmission at 410 mμ of the reagent blank as indicated in the procedure.

(e) Gum acacia solution (2% w/v): 2 g. of good-quality powdered gum acacia is dissolved in ammonia-free water in a 100-ml. volumetric flask and made up to volume. 1 ml. of CCl_4 is added as a preservative. The solution is clear and should be discarded when turbidity develops.

Procedure

Permanent color blank. The per cent transmission of the reagent blank is determined as follows: To each of five 30-ml. capacity micro-Kjeldahl flasks containing 10 ml. of ammonia-free water, 1 ml. of the digestion mixture and glass bead are added. The flasks are then placed in 50-ml. capacity Glas-Col heating mantles which are connected in parallel to a Variac so that the voltage can be maintained at 110 v. Their contents are digested exactly for 3 min. after white fumes appear. The digested mixture is cooled and diluted with ~ 30 ml. of deionized water. One milliliter of a 2% gum-acacia solution is added and the volume is made up to 50 ml. with deionized water. Two milliliters of Nessler's Reagent are added to 10-ml. aliquots from each flask and the samples are incubated at 23 ± 1° C. for 30 min. The per cent transmission is read in a Coleman Model 11 Spectrophotometer at 410 mμ with water as blank. A $K_2Cr_2O_7$ solution having a per cent transmission at 410 mμ equal to the average of these values is prepared and used as a permanent color blank in subsequent tests, adjusting its per cent transmission to a reading of 100.

Standard curve for nitrogen concentration vs. per cent transmission. Suitable aliquots of the standard ammonium-chloride solution are transferred into a series of micro-Kjeldahl flasks so as to contain ten levels from 0 to 0.18 mg. N. The volume in each flask is made up to 10 ml. with deionized water. The contents of the flasks are then digested and Nesslerized, and their per cent transmissions are determined as described under the preparation of the permanent color blank. From these results, the least-squares equation or the standard curve to be used in the estimation of unknown samples is obtained.

Analytical procedure. Exactly 1 ml. of milk (or 1 g. of milk if the weight basis is to be considered) is transferred into a 500-ml. volumetric flask and made up to volume with deionized water and mixed well. A 10-ml. aliquot of the diluted milk sample is transferred into a 30-ml. micro-Kjeldahl flask, and 1 ml. of the digestion mixture and a glass bead are added. The procedure used in establishing the standard curve is followed, except that digestion is continued for 3 min. after the solution fades from a dark brown to a pale yellow color. From the per cent transmission of the sample obtained at 410 mμ on the spectrophotometer, the amount of nitrogen present in the aliquot used for digestion is determined from the least-squares equation or the standard curve.

EVALUATION OF THE METHOD

Whole milk was sterilized in a Mallory small-tube heat-exchanger at 285° F. with a heating time of 4.16 sec., a holding time of 2.08 sec., and a cooling time of 6.24 sec. The sterilized milk was collected in 30-ml. sterile tubes and

TABLE 1

Comparison of the macro-Kjeldahl and modified direct Nesslerization methods for nitrogen in stored sterile milk

Days of storage	Macro-Kjeldahl method Digestion			Modified direct Nesslerization method Digestion 1			Modified direct Nesslerization method Digestion 2		
	1	2	Av.	1	2	Av.	1	2	Av.
	(mg. N/g of sterile milk)								
0	4.96	4.97	4.97	4.90	5.05	4.98	5.21	5.15	5.18
2	4.97	5.01	4.99	5.03	5.06	5.05	4.96	4.90	4.93
4	5.02	4.96	4.99	5.07	5.08	5.07	5.04	5.03	5.04
6	4.90	4.90	4.90	5.00	4.92	4.96	4.78	4.87	4.82
8	4.89	4.93	4.91	4.72	4.86	4.79	5.03	4.84	4.94
10	4.90	4.96	4.93	5.00	5.00	5.00	5.15	5.15	5.15
12	4.89	4.95	4.92	4.96	4.75	4.86	4.91	4.88	4.90
14	5.08	4.84	4.96	5.04	4.97	5.01	5.09	5.11	5.10
16	4.95	5.01	4.98	4.96	4.88	4.92	5.06	4.94	5.00
18	5.08	4.77	4.93	5.11	5.11	5.11	4.99	4.97	4.98
20	4.90	5.07	4.99	5.05	4.98	5.02	4.71	4.66	4.69
22	5.05	5.04	5.05	4.89	4.82	4.85	4.88	4.92	4.90
24	4.99	4.92	4.96	5.19	5.14	5.16	4.99	4.92	4.96
26	4.93	4.89	4.91	4.92	4.95	4.94	4.96	5.07	5.02

stored at 70° F. for a period of 26 days. Samples were analyzed on alternate days for their nitrogen content, both by the macro-Kjeldahl method (1) and by the modified direct Nesslerization method. Duplicate digestions of weighed samples from two different tubes of milk were made by each method. Duplicate Nesslerization of each digested sample was performed in the case of the modified micro-method. The results are given in Table 1.

Application of the Student's t test to these results indicates that there is no significant bias in the modified direct Nesslerization method for nitrogen when compared with the macro-Kjeldahl determination. Comparison of the error variance for the two methods indicates that the micro-nitrogen values are as reproducible as the macro-nitrogen values. The percentage standard deviation for a single nitrogen determination by the modified procedure is $\pm$ 2.0% of the nitrogen present. Although analysis of variance indicates a statistically significant effect of time upon the nitrogen content as determined by the micro-nitrogen method, possibly due to slight uncontrollable variations in digestion conditions from day to day, the variance expected from this source is sufficiently small that it can be included in the error term without seriously increasing the error variance. Therefore, the modified micro-nitrogen determination appears to be satisfactory for stored sterile milk analysis.

It has also been observed that the modified procedure can be used satisfactorily for the determination of nitrogen in difficultly digested samples, such as lipo-protein fractions and other high fat products, provided sufficient additional H_2SO_4 is added in 1-ml. aliquots to complete the digestion.

ACKNOWLEDGMENTS

The authors wish to acknowledge the assistance of Mr. K. S. Ramachandran and Dr. H. K. Wilson of this laboratory in preparing the sample of lipo-protein fractions and the sterile milk samples, respectively.

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A PROPOSED METHOD FOR DETECTION OF COLIFORM ORGANISMS IN COTTAGE CHEESE

While regulations pertaining to permissible levels of members of the coliform group in Cottage Cheese are found in many health codes, *Standard Methods* (1) does not specify a procedure for their enumeration. Since coliforms may be entrapped in the curd, and since curd particles vary greatly in size and firmness, the difficulty of arriving at accurate coliform estimates has led various laboratories to devise their own modifications.

In addition to the problem of insolubility of Cottage Cheese curd in sterile dilution water, the acidity of Cottage Cheese may be expected to lower the pH of the cheese/media mixture to levels lower than desirable. At the request of The Pennsylvania Approved Laboratory Directors Association, various concentrations of alkaline salts known to be capable of solubilizing milk proteins were investigated.

In the procedure, concentrations of alkaline salts as indicated in Table 1 were prepared as sterile dilution solvents, and the method evolved for coliform determination was as follows:

1. Sterilize a Waring Blendor container by suitable means. Autoclaving is suggested, but a complete rinsing with 70% ethyl alcohol, followed by rinsing with sterile water, will yield practical sterilization. Use of an aluminum mixing container with a screw top is suggested.
2. Aseptically transfer the entire contents of a retail package of cheese to the Blendor. Most satisfactory results have been found when 3/4 to 1 lb. of cheese is used.
3. Grind the cheese completely in the Blendor.
4. Accurately weigh 11 g. of the product into 99 ml. of a sterile 99-ml. dilution containing the protein solvent.
5. Shake the cheese-dilution mixture thoroughly and warm to 100° F. in a water bath, to melt any churned fat.
6. Since the resulting cheese-dilution mixture yields a 1/10 dilution, by splitting 10 ml. among three Petri dishes a total of 1 g. of cheese will be examined.
7. Pour approximately 15 ml. of violet red bile agar in each dish, mix, allow to harden, and cover with 3-4 ml. of the same media.
8. Incubate for 18-24 hr. at 35 or 37° C.
9. Count only typical dark red colonies at least 0.5 mm. in diameter.

Table 1 shows results obtained using disodium phosphate, sodium citrate, and sodium borate at varying concentrations as a Cottage Cheese solvent. Controls were run using sterile dilution water prepared in conformity with *Standard Methods* (1). Little difference was noted in the coliform counts obtained on these samples, with the exception of the rather substantial decrease when the dilution contained 0.5%

TABLE 1

Effect of varying concentrations of three alkaline protein solvents on pH of cheese dilution and cheese/dilution/media mixtures and on coliform counts per gram of cottage cheese. Average of ten samples except where noted

	Per cent concentration				
	0.2	0.3	0.4	0.5	0.6
	Disodium phosphate ($Na_2H\ PO_4$)				
pH cheese + dilution	6.14	6.38	6.59	6.71	.....
pH cheese + dilution + media	6.72	6.77	6.87	6.94	.....
Coliforms per gram	42	44	45	45	.....
	Sodium citrate ($Na_3C_6H_2O_7$)				
pH cheese + dilution	5.94*	6.03	6.11	6.14	6.13**
pH cheese + dilution + media	6.46*	6.65	6.67	6.70	6.70**
Coliforms per gram	35	42	39	39	41
	Sodium borate ($Na_2B_4O_7$)				
pH cheese + dilution	7.11	7.82	8.08	8.38	.....
pH cheese + dilution + media	7.01	7.22	7.38	7.52	.....
Coliforms per gram	42	38	37	26	.....
Average of controls					
pH cheese + dilution	5.32				
pH cheese + dilution + media	6.51				
Coliforms per gram	38				

* Av. of 4 samples.
** Av. of 6 samples.

sodium borate. From a practical standpoint, the use of 0.5% disodium phosphate solutions gave quick solubility, the pH of the mixture plus the media approached optimum levels, and the background color was normal. Excessively high pH reactions gave backgrounds with a yellowish color, as might be expected with neutral red.

The suggested procedure has the advantage of quickly and completely solubilizing the cottage cheese curd, and freeing organisms that may be trapped in curd particles. The resulting plates, using violet red bile agar as a medium, are very easily read and free from the usual curdy appearance obtained when cottage cheese is plated by the usual means.

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FATTY ACID REQUIREMENTS OF CERTAIN RUMEN BACTERIA[1]

Rumen fluid contains substances that stimulate growth of many bacteria which can not develop in the culture media usually employed for fastidious organisms. Some of the stimulatory factors in rumen fluid have been identified as fatty acids.

Bryant and Doetsch (4) have shown that *Bacteroides succinogenes*, a cellulolytic organism from the rumen, requires a combination of a straight-chain fatty acid and a branched-chain fatty acid. The straight-chain component can be either *n*-valerate or *n*-caproate, while the branched-chain requirement can be satisfied by either isobutyrate, isovalerate, or DL-*a*-methyl-*n*-butyrate.

Recently, the components of rumen fluid that are required by representative strains of *Ruminococcus flavefaciens* and *Ruminococcus albus* have been determined by Allison *et al.* (1). Again, the substances identified were fatty acids. However, these cellulolytic cocci from the rumen grew rapidly with a combination of acetate and either isobutyrate or isovalerate. Without acetate they required a much longer time to reach maximum numbers. The addition of *n*-valerate to the mixture did not further stimulate growth.

During the study reported here it was found that 20 of 90 isolates from the rumens of cows fed a high roughage ration would not grow in a rich basal medium (see below) unless rumen fluid were added. However, a mixture of fatty acids approximately equal in concentration to those found in rumen fluid would replace the material from the rumen. Five of these cultures were selected for further study and are the subject of this report.

The basal medium contained (in mg. per 100 ml.): trypticase, 500; phytone, 500; glucose, 200; resazurin, 0.1; cysteine HCl, 50; Na_2CO_3, 400; $(NH_4)_2SO_4$ and NaCl, 90; K_2HPO_4 and KH_2PO_4, 45; $MnSO_4$ and $CaCl_2$, 9. The medium was autoclaved and inoculated under a carbon dioxide atmosphere by Hungate's technique, as described by Bryant and Burkey (3).

Cultures were isolated originally from roll-tubes inoculated with 10^{-5} ml. of rumen material. The isolation medium was the basal mixture supplemented with 40% rumen fluid, 0.2% cellobiose, and 1.5% agar.

In some of the experimental trials, the basal medium was supplemented with the following mixture of fatty acids (in μM. per milliliter): acetate, 10; propionate, 2.3; *n*-butyrate, 1.3; isobutyrate, *n*-valerate, isovalerate, and DL-*a*-methyl-*n*-butyrate, 0.25 each; and *n*-caproate, 0.20. To study the effect of individual fatty acids or their combinations, branched-chain acids were used at a final concentration of 0.3 μM per milliliter of medium and straight chain acids at 0.6 μM per milliliter. These levels were found to be optimum by Bryant and Doetsch (4).

Growth was measured turbidimetrically in a Coleman Junior Spectrophotometer, Model 6B, at 500 mμ. The inoculum was 0.1 ml. of an actively growing culture in basal medium, supplemented with 40% rumen fluid or with valerate and isovalerate. Typical results are shown in Table 1. For each experiment a single inoculum preparation was used for the entire series; thus, the difference in growth for any one experiment was due to the fatty acids used in that series.

Culture A10 resembled the *Borrelia* species isolated by Bryant (2). His spirochete failed to grow without rumen fluid in the medium. Similarly, Culture A-10 failed to grow in the unsupplemented basal medium, but good growth was obtained when a combination of *n*-valerate and isovalerate was added. Only the branched chain acid was absolutely required, however. Cellulose digestion could not be demonstrated. Bryant's organism likewise failed to digest cellulose. In wet mounts, cells of A-10 appear to flex or lash in a manner typical of spirochetes. Cells are usually 0.3 to 0.5 by 7 to 9 μ. Gen-

[1] Published with permission of the Director, Wisconsin Agricultural Experiment Station.

TABLE 1

Effect of fatty acids on growth of selected rumen bacteria

Additive to basal medium	Culture number: A-10	2-3	2-13	1-8	A-2
	Maximum turbidity (O.D. × 100)				
None	0	0	0	0	0
n-Valerate and isovalerate	46	53	47	46	10
Isovalerate	22	11	4	17	11
n-Valerate	0	0	0	2	0
Fatty acid mixture	—[a]	—[a]	53	45	19
Time of readings (hr.)	24	35	30	24	60

[a] Not observed.

erally, there are three or four coils per cell and the cells occur singly or in short chains of three or four. The wave length averages about 2.5 μ and the amplitude about 1.2μ. Round bodies similar to those reported by Bryant are visible in older cultures. They average 3 μ in diameter.

Cultures 2-3 and *2-13* were identified as *B. succinogenes*. Microscopically, cells of Culture 2-3 often appeared ovoid, with conspicuous bipolar granules. Both of these cultures were actively cellulolytic. Good growth was obtained in the basal medium supplemented with a combination of *n*-valerate and isovalerate, as reported by Bryant and Doetsch (4).

Culture 1-8 was a Gram-positive rod usually found in pairs or short chains. The cells were 0.8 to 1.0 by 1.2 to 2.0 μ. Motility could not be shown. This culture grew well with glucose or cellobiose as carbohydrate sources, but digestion of cellulose could not be demonstrated. It produced carbon dioxide and hydrogen (analysis was performed with a Burrell gas analyzer). Most of the other products were neutral, for little drop in pH was observed. The taxonomic placement of this culture is not clear. As was true with Cultures 2-13 and 2-3, it grew well when isovalerate and *n*-valerate were added to the basal medium and to a limited extent with isovalerate alone.

Culture A-2 digested cellulose and used cellobiose as a carbohydrate source but not glucose. It was a Gram positive coccus that occurred singly or in pairs and produced white colonies in roll-tubes. Single cells were 0.9 to 1.1 μ in diameter. This organism has many characteristics in common with *R. albus* (5). Culture A-2 grew poorly in all media tried. However, it grew as well with isovalerate alone as with a combination of isovalerate and *n*-valerate. Slightly better growth occurred with the fatty acid mixture, possibly because of the acetate. Perhaps the substitution of Na_2S for cysteine would have increased the growth of this culture, as Allison *et al.* (1) have reported.

The growth stimulating properties of other fatty acids were tested on Cultures 2-13, 1-8, and A-2. They grew equally well if isobutyrate or DL-*a*-methyl-*n*-butyrate were substituted for isovalerate or if caproate were substituted for valerate.

This report, besides supporting earlier work (1, 4), mentions two additional strains of rumen bacteria (Cultures A-10 and 1-8) that require fatty acids. Culture A-10 appears to be a *Borrelia* species, and it requires a combination of isovalerate and *n*-valerate for good growth. Culture 1-8, a Gram-positive rod, has similar requirements. The digestion of cotton cellulose by these two cultures could not be demonstrated, whereas the three other cultures (2-3, 2-13, and A-2) were actively cellulolytic under the same conditions.

Experiments in progress confirm the observation that Culture A-10 has an absolute requirement only for the branched chain component. The straight chain acid (valerate) stimulates growth in the presence of limiting amounts of branched chain acid, but the straight chain acid alone will not support growth.

As suggested by Allison *et al.* (1), there appears to be mounting evidence that the unique requirement of rumen bacteria for branched chain fatty acids is not limited to one or two groups but extends to many members of this interdependent microbial population.

The isovalerate and *n*-valerate used in this work were analyzed by the silicic acid chromatography technique of Moyle *et al.* (6), modified to obtain better separation between valerate and isovalerate. The isovalerate contained 1 to 2% isobutyrate-butyrate (these acids are not separable by the method), whereas the valerate did not contain detectable amounts of any contaminating acids. It is unlikely that the trace amounts of isobutyrate-butyrate in the isovaleric acid significantly affected the results in the experiments reported here, because essentially the same results were obtained in other experiments in which the branched chain acids were completely free of detectable amounts of contaminating acids.

After this note was submitted for publication, Allison *et al.* (Arch. Biochem. Biophys., 84: 245. 1959.) showed that *R. flavefaciens* incorporated isovalerate-1-C^{14} into leucine of the cell protein.

SUMMARY

Cultures were isolated from 10^8 dilutions of rumen material in roll tubes of a trypticase-phytone-glucose basal medium supplemented with rumen fluid. Almost one-fourth of the isolates failed to grow on subculture in the basal medium, unless it was enriched with rumen fluid or a mixture of fatty acids commonly found in the rumen.

In further trials with five representative isolates, all cultures grew well with a combination of *n*-valerate and isovalerate replacing the rumen fluid or the fatty acid mixture. None grew with *n*-valerate as the sole additive to the basal medium, but all grew appreciably with isoval-

erate. Four of the five grew considerably better with a mixture of the two acids than with isovalerate alone. One of the cultures was tentatively identified as a *Borrelia* species and two as *Bacteroides succinogenes*. The two other organisms were not identified as recognized species.

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BLOAT VERSUS NO BLOAT IN IDENTICAL-TWIN COWS FED IDENTICAL RATIONS[1]

Smith *et al.* (3) reported that frothy bloat could be produced by feeding 16 lb. of a grain mixture and 4 lb. of unground alfalfa hay. It has since been found that a ration consisting of equal amounts of this grain mixture and hay would sustain the frothing condition once it had been initiated, but would seldom initiate frothy bloat in a normal cow. This fact permitted studying the composition of the rumen liquid from bloated and nonbloated cows independent of ration effects. During a 3-wk. preliminary period, one member of each of two pairs of identical twins was assigned to a ration consisting of 16 lb. of the grain mixture of Smith *et al.* (3), plus 4 lb. hay, while the other member of each pair of twins was given a ration of 20 lb. of hay. The ration of all cows was then changed to 10 lb. of the grain mixture plus 10 lb. of hay for a 2-wk. period, at the end of which samples of rumen liquid were taken via rumen fistulae equipped with plastic cannulae. The cows which had previously received the high-grain ration were bloating regularly at this time. The liquid was clarified by centrifuging for 30 min. at 15,000 r.p.m. in a Servall centrifuge. The short chain fatty acids, pH, sodium, potassium, and ammonia were determined in the clarified liquid. The short chain fatty acids for Twins 1 and 2 were determined at eight intervals relative to feeding, and the mean value is presented in Table 1. The samples from Twins 25 and 26 were taken 6 hr. after feeding. The other determinations were carried out on the dry matter prepared by concentrating the liquid at reduced pressure at 65°, followed by freeze-drying. The extractive and carbohydrates were determined by the method of Lagowski *et al.* (1). The lipide and nitrogenous components seemed to occur in higher concentrations in the rumen liquid from the bloated cows. The pH was lower in the bloated cows. It has also been

TABLE 1

Composition of clarified rumen liquid

Acetic Acid *(M %)*	67.4	67.3	64.0	67.2
Propionic Acid *(M%)*	17.6	15.9	19.5	16.8
Butyric Acid *(M %)*	11.2	14.6	11.7	13.9
Higher acids *(M %)*[a]	3.8	2.1	4.8	2.2
Total acids *(μM/ml.)*	114	129	104	172
pH	6.7	6.6	7.0	6.9
Ammonia N *(mg/100 ml.)*	18.2	11.4	15.4	14.3
Dry matter *(g/100 ml.)*	1.5	1.5	1.5	2.9
Ash *(% of D.M.)*	49.7	.....	48.9	.....
Benzene-Alcohol extract *(% of D.M.)*	2.7	2.5[b]	2.0	1.9[b]
Ether extract *(% of D.M.)*	1.8	0.3[c]	1.2	0.2[c]
Reducing sugars *(% of D.M.)*	0.4	....	0.2	....
Nonreducing sugars *(% of D.M.)*	0.0	....	0.2	....
Starch *(% of D.M.)*	0.9	....	0.7	....
Acid hydrolyzable polysaccharides *(% of D.M.)*	1.8	....	1.6	....
Crude protein *(% of D.M.)*	4.1	....	3.0	....
Sodium *(mg/100 ml.)*	295	....	304	....
Potassium *(mg/100 ml.)*	88	....	86	....

[a] Water-soluble acids with a higher mobility than butyric acid on silicic acid columns.
[b] Direct 80% ethanol extraction of dry matter.
[c] Direct hexane extraction of dry matter.

[1] Published with the approval of the Director of the Michigan Agricultural Experiment Station as Journal Article No. 2568 and partially supported by funds from the North Central Regional Research Project No. 27.

observed by Phelps (2) that a larger amount of magnesium ammonium phosphate containing precipitate could be isolated from the rumen liquid of T-26 (bloated) than from T-25 (nonbloated). The role these differences in chemical composition play in the etiology of bloat remains to be determined.

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INTERPRETIVE SUMMARIES OF PAPERS

IDENTIFICATION OF VOLATILE CARBONYL COMPOUNDS FROM CHEDDAR CHEESE

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Cheddar cheese flavor is very complex and results from the enzymatic breakdown of proteins, carbohydrates, fat, and other milk constituents during ripening of the cheese. It normally requires up to 1 yr. to obtain the desired Cheddar flavor. This ripening time is costly because of the large inventory that cheese plants must carry. Furthermore, cheese is an active system and even with careful control of ripening conditions, inferior flavored cheeses frequently occur. Interest in the chemical identity of Cheddar flavor stems from a desire to shorten curing time, to provide better control of the ripening process, and to provide adequate objective methods for measuring quality.

The carbonyl compounds are one of a number of families of compounds that are found in the volatile portion of cheese flavor. Some earlier work in this laboratory suggested that certain members of this family of compounds might be of major importance in Cheddar flavor. It was the purpose of this investigation to obtain further information on the volatile carbonyl compounds in Cheddar cheese.

It was necessary to use large quantities of cheese in order to isolate sufficient quantities of carbonyl compounds for identification. Forty-pound blocks of Cheddar were made into a water slurry and distilled at low temperature to remove the carbonyls. The carbonyls were then selectively isolated from the distillate by reaction with 2,4-dinitrophenylhydrazine, a carbonyl condensing reagent. The hydrazone derivatives were extracted, concentrated, the mixture separated by chromatography, and the individual purified derivatives identified.

Some of the carbonyl compounds would appear to originate from milk fat, whereas amino acids probably serve as a precursor of others. Methional, a sulfur-containing aldehyde, was identified in Cheddar cheese. This compound exhibits a cheese-like odor at the proper concentration. It is very potent (low flavor threshold) and may contribute to flavor when at trace levels of concentration. The most logical source of methional is the amino acid methionine.

The approximate concentration of the identified carbonyl compounds was determined and synthetic mixtures were then evaluated in simulated Cheddar cheese systems to determine their importance to Cheddar flavor. It was concluded that the carbonyl compounds contribute to the flavor, but the proper balance of all compounds, known and unknown, would appear to be critical in Cheddar cheese flavor.

COCONUT-LIKE FLAVOR DEFECT OF MILK FAT. IV. DEMONSTRATION OF δ-DODECALACTONE IN THE STEAM DISTILLATE FROM MILK FAT

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Sterilized or dried milk products containing butterfat undergo flavor change during processing and storage. This change originates to a significant degree in the butterfat and renders such products readily distinguishable in flavor from fresh milk. Although undesirable in beverage milks, this same type of change imparts the pleasant and unique flavor that is derived from cooking and baking with butter.

The identification and measurement of flavor compounds involved in this change have been undertaken to provide a basis for control of the phenomenon. The problem involves the isolation and chemical characterization of compounds which are generated only to the extent of parts per million in milk products. Previous efforts accomplished the identification of δ-decalactone, a compound with potent buttery-coconut-like flavor quality, in dried whole milk, dried cream, evaporated milk, and heated butterfat. This compound is absent in unheated fresh products.

The present investigation has enabled identification of δ-dodecalactone, a compound having a strong peach-like flavor quality. It is closely related in structure to δ-decalactone and appears to be produced in smaller quantities than the latter lactone when milk fat is heated.

A sensitive gas chromatographic procedure for measuring these lactones has been devised. An attempt is being made to adapt this method for screening milk supplies regarding their potential to develop lactones.

EFFECTS OF LACTIC CULTURES ON ACIDITY AND FIRMNESS OF COTTAGE CHEESE COAGULUM

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The strength of Cottage cheese curd varies with different cultures used in making. This is such an important relationship that this study was made to observe the effects of 17 different cultures on curd strength, titratable acidity of whey, and pH of the coagulum. The term coagulum is used here to indicate any curd formation before cutting, ranging from the first evidence of weak gel formation to extreme hardness.

For uniformity in all trials a single lot of nonfat dry milk (NDM) was used; it was always reconstituted by mixing 11 lb. with 89 lb. of water. For testing each culture, 1,200 ml. of the skimmilk was warmed to 90° F. and mixed with 85 ml. of the culture to be tested. After 30 min., rennet was added at the rate of

1 ml. per 1,000 lb. of skim. Then the mixture was divided equally between six beakers which were covered and held at 90° F. One beaker was carefully observed for the first signs of coagulation, and when that occurred the time was noted and measurements were made as quickly as possible of curd strength, pH, and titratable acidity of whey. These observations were repeated at intervals on the other five beakers to obtain measurements over wide ranges of acidity and curd strength. Curd strength was measured by the Cherry-Burrell curd tension meter, pH was measured at 90° F. with a Beckman Model G pH meter and glass electrode.

Curd strength at any given titratable acidity varied widely between the different cultures. Some cultures developed curd with strength values over 100 g. at 0.55% titratable acid in the whey; other cultures failed to coagulate the skimmilk until the acidity had exceeded 0.55%.

Curd strength at any given pH value did not vary as much between different cultures as it did with titratable acidity measurements. Actually, differences between cultures were remarkably small when curd strength was observed at identical decreases in pH after coagulation.

When the 17 cultures of this study were examined it was found that the relationships between titratable acidity of whey and pH of curd varied with every culture. For instance, with pH 4.75 in the curd, the titratable acidity varied from 0.57% for Culture P to 0.76% for Culture W. When one culture produces a lower titratable acidity than another culture when both have the same pH value, it is probably because the second culture produces a greater proportion of weak acids, such as carbonic or acetic acids. Undoubtedly, for this same reason, certain cultures which developed high titratable acidities at the A-C end point of about pH 4.65 also produced curd which floated when heated. Such undesirable cultures for Cottage Cheese would be expected to possess the high aroma desirable in making cultured buttermilk.

The relatively close relationship between curd strength and pH of curd, and the rather poor relation between curd strength and titratable acidity of the whey, suggest that pH is probably the more reliable index for controlling Cottage cheese operations. These relationships also explain why occasional vats of skimmilk for Cottage cheese do not coagulate even when the titratable acidity of the milk is as high or higher than that normally expected to cause coagulation.

A PROPOSED METHOD FOR DETECTION OF COLIFORM ORGANISMS IN COTTAGE CHEESE

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Lack of a standard procedure for the enumeration of coliform organisms in Cottage Cheese prompted The Pennsylvania Approved Laboratory Directors Association to request evolvement of an acceptable determination for these organisms.

The problem of coliform recovery from Cottage cheese curd may be complicated by entrapped organisms in the curd. In addition, since Cottage Cheese

curd may show an acid reaction, the pH of the curd-media mixture in the determination may be lower than desirable. To solubilize Cottage Cheese curd and to adjust the pH of the curd-media mixture to levels acceptable for other dairy products, various alkaline protein solvents were investigated. Possibly the most successful was a sterile 0.5% disodium phosphate solution.

The procedure for enumeration of coliform in Cottage Cheese, as suggested, involves grinding the curd in a sterile Waring Blendor, followed by weighing 11 g. of the product into 99 ml. of sterile 0.5% disodium phosphate solution. The mixture is thoroughly shaken and warmed to 100° F. to facilitate solubilization of the curd and to melt any churned fat. Plating, using violet red bile agar as the medium, then can be done as usual. The resulting plates will be found to be free of curd particles. The use of the alkaline solvent adjusts the reaction of the curd-media mixture to pH 6.9–7.0.

UTILIZATION OF VARIOUS SUGARS BY THE YOUNG DAIRY CALF

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The high cost of fluid milk has encouraged the use of milk replacers in the diet of dairy calves, although most milk replacers contain large amounts of milk products

This investigation was undertaken to study the ability of the young dairy calf to utilize certain sugars when included in the diet as possible economical sources of energy. The purpose of feeding such sugars was to determine whether such energy sources could be used to replace a large proportion of the energy in the calf's diet which normally is supplied by milk or milk products.

Many studies have shown that the young calf (1–8 wk. of age) is unable to utilize starch. More recent work has shown that young calves have little or no invertase (enzyme) to aid in the inversion of sucrose (granulated sugar) to glucose, a simple sugar. Some workers agree that glucose may be utilized by young calves, whereas others have reported only slight utilization.

In this study, male dairy calves, ranging in age from 1 to 14 wk., were studied to test their ability to utilize chemically pure sugars, namely sucrose, fructose, glucose, lactose, galactose, and maltose as measured by changes in total reducing substance (TRS) levels which are largely glucose in the blood. Blood samples from calves were taken at regular intervals over a 4-hr. period following the oral administration of the sugars. The sugars were fed following a 24-hr. fast at the rate of 2 g. per pound of body weight. The following sugars: glucose, lactose, and galactose gave marked elevations in blood TRS with no evidence of diarrhea. Calves were unable to utilize sucrose, while the uptake of fructose and maltose was comparatively low. Feeding these sugars was accompanied by diarrhea. Sucrose inverted (changed) by the enzyme invertase, or citric acid was readily utilized as shown by elevations in blood TRS.

BLOOD AND RUMEN CHANGES FOLLOWING THE INTRA-RUMINAL ADMINISTRATION OF GLYCOGENIC MATERIALS

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Lactic acid accumulates in the rumen after feeding certain rations and much is fed in good-quality silages. This lactic acid might be absorbed from the rumen or converted to other acids by rumen bacteria. There is little evidence in the literature to indicate the extent of absorption, but considerable evidence to indicate that much rumen lactic acid is converted to other acids. The relative magnitude of the formation of acetic, propionic, and butyric acids from lactic acid usually has been indicated in the literature by stating that primarily propionic acid is produced. The ratios of the three fatty acids produced from lactic acid may be important influences on the efficiency of ration utilization or relative ketogenic effects. Whether ruminal butyric acid contributes to blood ketones or blood glucose is somewhat controversial in the literature. The availability of lactates from the cheese industry has resulted in a consideration of them for ketosis therapy.

This work was initiated to determine the fate of lactates in the rumen as indicated by the proportions of the three acids produced and the magnitude of ruminal absorption. Ratios of propionite and butyrate, the acids of which were produced in the greatest quantities and have previous indications of either strongly favoring glucose or ketone production, were infused into the rumen to measure effects on blood ketones and glucose. Sodium propionate, propylene glycol, a three-carbon unit, and sucrose were given to compare their effects on the rumen acids and blood glucose and ketones with those of lactates.

Bacterial conversion of lactate to fatty acids gave ruminal increases in molar ratios of propionic, 0.86; butyric, 0.51; and acetic, 0.37. The percentage loss of lactate by absorption increased as rumen concentrations increased. Below 200 mg. % lactic acid in the rumen, which is in the range of maximum reached on normal rations, most of the lactate appeared to be converted to fatty acids. Ruminal infusion of propionate-butyrate mixtures increased blood glucose and decreased blood ketones as the proportion of propionate increased. On an equal-dose basis, the compounds may be ranked from greatest to least response on basis of blood glucose as follows: propylene glycol, sodium propionate, lactates, and sucrose. Although all of these materials should increase blood glucose if given into the blood, rumen bacteria alter some of them more than others when they pass into the rumen.

COMPARISON OF DAIRY SIRE PROGENY TESTS MADE AT SPECIAL DANISH TESTING STATIONS WITH TESTS MADE IN FARMER HERDS

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In 1945, the first dairy sire progeny testing stations in Denmark were established. During the testing year 1945-46, six sires were tested, two at each of three stations. In 1956-57, 94 sires were tested and were distributed among 33 stations. For the 12 testing years, 1945-46 to 1956-57 inclusive, 547 sires have been tested. By visiting the test stations and talking with Danish farmers it was apparent that they were popular and were of much interest to breeders.

In recent years, the feasibility of establishing dairy sire progeny testing stations in the United States has been considered and some groups have expressed interest in such stations. The purpose of this study was to determine how precisely the production of future daughters in farmer herds could be predicted from tests made at special test stations, and to compare the predictive value of tests at test stations with that of tests in farmer herds.

One of the most striking results of the study was the relatively large, between, and within sire components of variance for tests at the test station. The components of variance between sires at the same station in the same year were 107,652 for milk and 229 for butterfat. The corresponding components for the same sires tested in farmer herds were 32,125 and 53. The within sire components of variance for the test stations were 541,696 and 1,280 for milk and butterfat, respectively, while those for tests in farmer herds were 527,477 and 916, respectively. With these components, heritability of milk and buterfat were found to be 0.66 and 0.61, respectively, for test station data, and 0.23 and 0.22 for tests in farmer herds. It was concluded that part of the inflation of the between sire components for test station data was caused by environmental effects becoming coufounded with the progeny groups.

The regression of the production of a future daughter in a farmer herd on the tests in farmer herds was higher than that of the future daughter in a farmer herd on the tests at test stations. This was true for both milk and butter production for progeny tests based on five or more daughters. The progress expected from selection is proportional to the correlation between the characteristic selected for and the criterion used as a measure of that characteristic. Using tests at test stations and tests in farmer herds as criteria for measuring the production of future daughters in farmer herds, it was found that for milk production the tests in farmer herds were superior to tests at test stations in all cases, except where the number of daughters comprising the test was five or less. For butterfat production, the tests in farmer herds were superior for all cases, except where the number of daughters comprising the test was 15

or less. More genetic progress per generation could result from the use of test stations if more sires were tested with fewer daughters per sire.

It was concluded that in areas where relatively large percentages of cows are on test, there is little need for test stations. On the other hand, where only small percentages of cows are on test, there is a need for test stations. Assuming that test stations can be designed so as to prevent environmental factors from becoming confounded with sire groups, the main limitations are the number of sires that can be tested and the costs of operating the stations.

PROTEIN-BOUND IODINE IN THE BLOOD OF DAIRY CATTLE FED A TRACE-MINERALIZED SALT

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The milk production of cows in the Southern United States is affected by summer and fall weather conditions. In spite of good feeds, milk production is very difficult to maintain in the fall and could be a carry-over effect of summer. Since it is recognized that the thyroid glands of cows in the southern states are smaller than those of similar background in the North, and since protein-bound iodine (PBI) has been thought to be a possible measure of the thyroid activity, this experiment was conducted to study the relation of PBI to milk production or growth. A trace-mineralized salt containing iodine from cuprous iodide and 3,5-diiodosalicylic acid was fed routinely as 1% of the concentrate.

The relation of PBI of milking cows to the stage of lactation, season of year, and breed was studied. The PBI of milking cows averaged 15.62 γ per 100 ml. at 30 days postpartum, 13.34 at 150 days, and 10.93 at 270 days. The PBI averaged 24.13 γ per 100 ml. for Holsteins, 19.89 for Brown Swiss, 15.81 for Jerseys, and 15.16 for Guernseys. The range of PBI values for all milking cows was 4.69 to 44.28 γ per 100 ml. Highly significant differences in PBI levels of milking cows were found in respect to stage of lactation, breed, and season of year. The PBI was higher in spring and summer, declining to a low in January. Correlation coefficients between PBI and 4% FCM produced were positive for all breeds and significantly so in most of the comparisons.

The PBI levels of calves and heifers varied widely, ranging from 2.02 to 46.67 γ per 100 ml. of plasma. With calves and heifers the differences were significant for breed, highly significant for seasons, and nonsignificant for age. In general, the plasma PBI levels decreased with age. No significance relationship was found between PBI and growth as measured by increase in height at withers.

Shortly after the experiment began it was evident that the PBI values were higher than those found in most other experiments. An experiment was conducted to study the source of iodine in the salt as possibly causing elevated values. Neither plain salt nor salt containing KI caused an elevation; but when the salt containing cuprous iodide and 3,5-diiodosalicylic acid was fed, the blood values

increased. Work at West Virginia has indicated that the feeding of grass silage to their herd caused elevated PBI values.

In spite of the high plasma PBI levels found during the study, differences for stage of lactation, season of year, and breed and the relation to milk production or growth were found. Upon theorizing, it had been expected that the low PBI values would be in the fall. The lowest values were found in January.

INVESTIGATION OF THE OXIDATION-REDUCTION POTENTIAL OF RUMEN CONTENTS

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The rumen, due to its important digestive functions, has been studied extensively in the past 20 yr. by workers in the fields of bacteriology, biochemistry, and dairy science. These studies have resulted in the characterization of a group of parameters (e.g., acidity, ammonia production, volatile fatty acid production, cellulose digestion, and bacterial counts) which define the normal state. These parameters have diagnostic value when a metabolic disturbance is noted, in that a deviation from the normal in one or several of these parameters may indicate the cause of the disturbance. The purpose of this investigation was to characterize another parameter, oxidation reduction potential, which could aid in understanding the rumen process.

The measurement of the oxidation-reduction potenetial is carried out by connecting a platinum electrode, which is in the solution to be tested, through a very sensitive volt meter to a standard hydrogen electrode. The potential of the two electrode systems is different and electrons will flow from one to the other. The force of this flow is measured with the volt meter. The reading on the volt meter is then taken as a measurement of the oxidation-reduction potential of the unknown solution.

The results obtained in the first part of the investigation indicated that a very clear-cut relationship exists between metabolic rate of rumen microorganism and oxidation-reduction potential. The potential observed in rumen contents removed from cows maintained on a hay-grain diet was lower than that observed in cows fed hay alone. Since it is well-known that grain is digested much more quickly than hay by rumen organisms, it may be concluded that the potential was lower in cows fed grain because the metabolic rate of the rumen organisms was higher. Other results which further justify this conclusion were obtained by measuring the potential at several different times during the day. The observed potential was low for several hours after feeding and then slowly increased until the next feeding, at which time it decreased again. The slow increase in potential after feeding is due to a disappearance of readily available nutrients from the rumen, causing a decrease in the metabolic rate of the organisms.

The second phase of the experiment involved the derivation of a mathematical formula relating the oxidation-reduction potential to acidity. Such a relation-

ship is required in order that one may compare the potentials observed in rumen fluid from different sources on a common basis.

STUDIES ON 9α-FLUOROPREDNISOLONE ACETATE, A NEW SYNTHETIC CORTICOSTEROID FOR THE TREATMENT OF BOVINE KETOSIS

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Bovine ketosis is still not completely understood, but in recent years notable advances have been made in its treatment. In 1950, cortisone and hydrocortisone were first used successfully in the treatment of this condition. Following the use of these natural glucocorticoids, a number of synthetic glucocorticoids have appeared on the market for this use. The development of these newer, more potent, and more specific steroids for use in human medicine has made them available and practical to veterinary medicine.

9α-Fluoroprednisolone acetate is an example of one of these synthetic compounds. In the rat this compound was about 50 times as potent as hydrocortisone as a gluconeogenic agent and 14 times hydrocortisone as an anti-inflammatory steroid. These qualities made this steroid a likely candidate for treatment of bovine ketosis.

To verify the rat data, and to establish an effective dose, experiments were carried out in normal cows to determine the relative potency of 9α-fluoroprednisolone to other steroids such as prednisolone, which are currently used for the treatment of ketosis. Also, experiments were carried out to determine the limits of safety for the use of 9α-fluoroprednisolone acetate in cows under different circumstances.

Using the degree of elevation of blood glucose as the means of comparison, it was found that 10 mg. 9α-fluoroprednisolone acetate would produce the same response that required 100 mg. prednisolone. Administration of repeated doses of 9α-fluoroprednisolone resulted in elevated blood glucose levels without causing untoward effects. High doses of 9α-fluoroprednisolone, 100 mg., were given safely on consecutive days, producing a relatively long-lasting elevated blood glucose. At these high dose levels, some depression of the serum potassium was observed when blood was analyzed chemically. However, no signs of potassium deficiency were manifested in the cows.

The oral use of sodium propionate concurrently with the intramuscular use of 9α-fluoroprednisolone caused some transient clinical signs of depressed serum potassium. Consequently, caution should be exercised if the two therapies are to be used concurrently.

9α-Fluoroprednisolone has been used with gratifying results in the treatment of clinical cases of primary bovine ketosis. These results will be the subject of a future paper.

FATTY ACID REQUIREMENTS OF CERTAIN RUMEN BACTERIA

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Studies of the bacteria in the bovine rumen repeatedly showed that lower colony counts were obtained when the cows were fed roughage than when they were fed mostly grain concentrate. Moreover, it was noticed that many rumen bacteria would grow in a culture medium containing rumen fluid but not in a rich organic medium without rumen fluid. Others have shown that some rumen bacteria will grow in an otherwise unsatisfactory medium on the addition of certain straight and branched-chain fatty acids. Therefore, it was decided to see whether organisms that seemed to require rumen fluid could be made to grow in its absence.

Five organisms obtained from cultures in rumen fluid agar would not grow when transferred to unsupplemented trypticase-phytone medium (a rich organic broth containing digested milk and soybean proteins plus other nutrients). Good growth occurred, however, when the medium was enriched with a mixture of *n*-valeric and isovaleric acids. Four of the five cultures also grew with isovaleric acid as the only additive, but not as well as with the mixture.

This work is of interest, in that it suggests a method of replacing rumen fluid in culture media. Rumen fluid is undesirable because its composition is unknown and it varies from sample to sample. Furthermore, rumen fluid is not always readily available to the bacteriologist.

OUR INDUSTRY TODAY

POLICIES AND PROGRAMS CONCERNING ANTIBIOTIC AND PESTICIDE RESIDUES IN MILK[1]

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It is a real privilege and a pleasure to represent the Food and Drug Administration here today. It means much to me personally to be able to take part in this conference under the leadership of Dean Palm, a college classmate and long-time friend. Food and Drug Administration officials have come to know Dr. Palm as an outstanding scientist with a constructive approach and a high regard for the broad public interest. The State of New York is to be congratulated for having named him the Dean of the College of Agriculture of this great university.

The Federal Food, Drug, and Cosmetic Act of 1938, at the time of its passage, stood as a landmark in modern food regulation. However, in the short span of 20 yr. a number of major amendments have been designed to keep the law in step with rapid technological developments. The last two such amendments were the Pesticide Amendment of 1954 and the Food Additives Amendment of 1958. These recognized the spectacular growth of the chemical industry during the law's life span, which had created the need for modernizing the law so that useful chemicals could be put to work once their safety was established.

Since 1939, sales in the chemical industry have increased fivefold and the industry today stands fourth in size among the industries of the country. It has produced hundreds of thousands of chemical entities, more than 10,000 of which are manufactured for commercial use. In today's world, the chemist has overnight changed his position from one of servant to that of master. To appreciate the validity of that statement, one has only to view the magnitude of the problem of developing adequate analytical methods and find a countless number of complex organic compounds in use today, or proposed to be used in or on food.

The research contributions of the drug and insecticide branches of the chemical industry, so great in the last two decades, have had a profound effect on world health and economic gains. Outstanding among the drug achievements has been the mass production of penicillin accomplished in World War II, and later the discovery and mass production of other life-saving antibiotics. Notable among insecticide achievements was the mass production of DDT beginning in World War II and since that time the discovery and production of a large number of other very useful organic pesticides. While the health and economic gains from these developments have been tremendous, we dare not let this blind us to their potentialities for harm.

The unfortunate but grim facts are that these truly great weapons against disease and pests are two-edged swords. Indeed, their ability to injure us exists just as certainly as does their ability to help us. It is ironic that the same two examples of spectacular achievements in the health and economic field, penicillin and DDT, in the main have made it necessary that we meet here to discuss ways and means of preventing their misuse, with resultant public harm. The two governmental agencies primarily responsible for the proper and adequate labeling of these commodities under the laws they enforce have been mindful of the potentiality for their misuse.

The U. S. Department of Agriculture enforces the Federal Insecticide, Fungicide, and Rodenticide Act of 1947. All economic poisons must be registered with the Department before they are shipped in interstate commerce. Following the directions for use on labels of pesticides thus registered should yield crops with residues within the tolerances set by the Food and Drug Administration. Growers of agricultural commodities have one simple rule to follow—use pesticides according to label directions—on the crops or kinds of animals specified, in the amount specified and at the time specified. The Food and Drug Administration enforces the labeling provisions of the Federal Food, Drug, and Cosmetic Act, which require, among other things, adequate directions for proper use and warnings against misuse in the labeling of drugs. Accordingly, pesticides and drugs in interstate commerce coming into the hands of farmers and dairymen for their use contain the kind of label instructions and warnings that, if followed, will produce an uncontaminated milk supply. Specifically, U. S. Department of Agriculture registered labels for DDT preparations warn against use of the chemical around dairy animals and against feeding treated forage to dairy animals. The label-

[1] Presented at the Dairy Industry Conference, Cornell University, Ithaca, New York, January 5, 1960.

ing of penicillin preparations for the treatment of mastitis warns milk producers to reject milk from treated animals.

What is the resultant health and regulatory problem if these articles are misused? Since the days around the year 1906, when formaldehyde was used to preserve milk, it has been the consistent policy of food and health officials generally to oppose the addition, in any amount, of poisonous or deleterious substances to milk. This philosophy still prevails.

Recently, a scientific advisory committee studied a proposal that residues of methoxychlor be permitted in milk because methoxychlor was proposed for use on dairy animals. After the study, the scientists recommended against the proposal. The Committee felt, in view of the importance of milk in the diet, that a greater margin of safety must be established in fixing a tolerance for pesticides in milk than would be the case for any other food in the human diet. Since there is no legal tolerance for any pesticide residue in milk, the interstate shipment of milk containing such a residue is illegal under the Act and the milk itself is subject to seizure.

If additional research should show that it is possible to set a tolerance for pesticide residues in milk without endangering the public health, the Food and Drug Administration will reconsider its present policy. As it stands now, pharmacologists are unable to point to any pesticide level of DDT and some of the other pesticides in milk which they would consider as safe for the infant, the elderly, and the sick, whose principal diet may consist of milk.

Residues of antibiotics in any amount also are illegal in milk. Such residues result from a failure, through ignorance or through deliberation, to reject milk from treated animals. In making this statement I am not unmindful of rumors, thus far unconfirmed, of the deliberate addition of penicillin to milk to lower bacterial counts. While it is, if true, a part of our general problem with which we must deal, we shall not dwell on it here. You and I can pretty well predict the fate of any operator convicted in the courts on a criminal charge involving such conduct. Aside from the matter of penicillin residues, I should point out that the Act also outlaws milk from sick animals; therefore, penicillin-contaminated milk from treated cows has two counts against it.

The Food and Drug Administration asked a group of internationally known physicians to consider the health problems arising out of antibiotic residues in milk. It was their conclusion that while there should be no antibiotics in milk, penicillin presents the major health problem. It was concluded by the panel that allergic reactions ranging from mild to fatal may occur from drinking milk contaminated with penicillin.

The problem being pretty well defined, how did we approach it? First, believing that the educational approach is a basic part of a sound regulatory program, in cooperation with the Extension Service of the U. S. Department of Agriculture, the National Milk Producers Federation, and others, we undertook a widespread campaign of education of the dairy farmer, both in methods to aid in the prevention of the occurrence of mastitis and in the use of penicillin in treating mastitis when it does occur. In the latter circumstances, our efforts were directed primarily at education as to the need for discarding milk from treated animals for an appropriate period of time following the last treatment with penicillin.

We took another look at the labeling requirements on mastitis preparations and amended the antibiotics regulations to increase the conspicuousness of requirements for discarding milk in the labeling of penicillin products used for the treatment of mastitis. Later, we took steps to limit the amount of penicillin contained in doses for intermammary infusion. This limitation was placed at 100,000 units per dose, a significant change from the previously used preparations which contained as much as 1,500,000 units per dose.

We allowed a reasonable time for the effects of these various steps to be noticeable. In the fall of 1958, we made a four-month investigation of market milk in the territories of sixteen of our field district offices. Our setup was such as to give uniform sampling across the country.

Of the 1,170 samples thus obtained and examined for penicillin, 3.7% were positive. This justified the conclusion that our educational activities had resulted in a noticeable reduction in the occurrence of penicillin in the milk samples in 1958, as contrasted with samples from the 1955 and 1956 surveys. Unfortunately, however, it does not demonstrate that the problem has been solved by educational means alone, nor are we now able to conclude that it will be solved.

While this work on antibiotics in milk was being carried on, we were at the same time obtaining data on pesticide residues in market milk. In 1955, we conducted a nationwide survey. Because we were in the process of developing analytical methods, the results on most samples were only semiquantitative. However, quantitative chemical tests on 169 samples believed to have highest insecticide residues (based on earlier bioassay) showed 33 samples with residues ranging from 0.05 to 1.5 p.p.m. of DDT or its equivalent.

In the same manner, we undertook to promote an educational campaign to aid in safer use of pesticides on dairy farms. We again had excellent cooperation from the U. S. Department of Agriculture and from the states and the milk industry associations. In the 1958 survey, 936 samples of milk were examined for pesticide residues, and 2.5% were found to

contain significant residues. These included DDT, DDD, Toxaphene, Chlordane, and BHC. Again, we were forced to conclude that educational measures are a great help but have not proved to be a complete remedy.

The Food and Drug Administration has developed a practicable and a more rapid test for the detection of antibiotics in fluid milk than we have heretofore been able to offer. Instead of taking a day or two to complete an examination of a single sample, we can now produce results in about 2.5 hr. The method has been furnished to state and city regulatory officials.

The use of this test should cause no more difficulty in industry and state and federal control than the various bacteriological tests which are routinely performed in your laboratories. It is true that evaluating the results of these inhibitory tests requires some training and skill, but they are not difficult. Essentially, the method involves previously used principles, but results are achieved much more quickly.

Chemical methods for pesticide residues have not been improved as much in simplicity of operation or speed. Available methods can be run by competent chemists with properly equipped laboratories, and we can and will supply references to the methods in use on request.

We are now organizing a series of schools to teach interested state and city regulatory officials both the pesticide and penicillin methods. To the extent that our facilities will permit, key industry representatives will be included in this educational program.

You may have some concern at the idea of having to examine as a part of production samples for penicillin and pesticide residues from each lot of milk received in your plants. We have made no such recommendation and perhaps it will not have to be done. Every fluid milk establishment has field men. The amount of information field men possess as to farm practices—not only on the farms of their own patrons but many times on the farms all around the countryside—is legendary. These field men know which milk producers are having trouble with mastitis, and we would think that in most instances they could guide plant management in sampling operations on suspect milk. We doubt that performing this function would impose any particular burden on field men, or be anything out of line with their normal operations. This is, of course, the negative aspect of the field man's operation. His principal objective in this area should be the carrying on of an educational program that will prevent contamination.

Field men may not be able to serve as reliable guides to sampling for pesticide residues. They can do a great deal as educators, to help be sure that the patrons are using pesticides properly in the dairy and on the cows, and are taking such precautions as they can to avoid the feeding of forage contaminated with pesticides. We recognize that it is quite a problem for farmers who purchase feed in open markets, to be sure that it does not contain residues of pesticide chemicals that may contaminate milk. Both state and federal checking on this phase of the problem is being accelerated. From recent discussions we have had with State officials engaged in enforcing feed laws, pesticide laws, residue laws, and State food laws, it is abundantly clear that there is a marked increase in the interest at the state level in the problems of contamination of foods and feed stuffs with pesticide residues. We are told that state laws are being strengthened and more and better facilities for the handling of programs in this area are being sought through increased funds from the state legislatures. It will be the purpose of the Food and Drug Administration, as a part of its general program of working closely with state and local organizations on mutual problems, to assist and encourage these officials in such programs. We will be glad to be advised of interstate supplies of dairy feeds which appear to be contaminated with pesticides. Appropriate action under the Act will be taken when evidence of contamination can be confirmed.

We have repeatedly emphasized that accepting and shipping milk which is contaminated with antibiotic or pesticidal residues can lead to serious trouble for all. How do we and you convince all interested persons that this is so? As a regulatory agency, once we have carried out an educational program that seems reasonable in content and extent, and find it has been only partially successful, we have left only the remedy of the application of the sanctions provided by the law. As you can appreciate, this sometimes serves as the most eloquent kind of encouragement for compliance and, unfortunately, many years of experience have shown this kind of encouragement to be necessary in a great many situations, in order to get the job done.

Inspectors of the Food and Drug Administration are now investigating the production of fluid milk in various areas of the country. Samples are being collected for examination for antibiotic and pesticide residues from shipments in interstate commerce or offered for interstate shipment. The samples are being examined by us, using the procedures that I have mentioned earlier, with identification specifically of penicillin where positive results are obtained. Where our examinations of such samples show the presence of penicillin or pesticidal residues, it will be our purpose to institute appropriate legal actions as provided by law. Something over a week ago 60 samples had been examined and found negative for pesticides and one sample was found positive for penicillin. The shipper has been cited to a hearing and a further

investigation of his milk supply is under way. As yet no seizures have been made. This regulatory program carried on by us has the definite objective of eliminating antibiotic and pesticide residues from milk to every extent that we can, in our resources, bring about such a result.

Our jurisdiction is limited to interstate commerce and, obviously, the problems can not be solved by regulation only of interstate milk. As indicated, we are discussing our program with state and local milk control officials, and we hope that we will be able to see concurrent steps being taken by them to apply controls to adulterated milk which does not enter channels of interstate commerce.

Corrective actions by regulatory officials, while a basic part of any program such as this, will not solve the problem. It is of such magnitude and complexity that the combined resources of all concerned, vigorously pursuing both preventive and corrective programs, is essential if real success is to be achieved. The Food and Drug Administration, to the extent of its facilities and consistent with its over-all obligations, pledges its full support to educational institutions and related groups in their efforts to reach that common goal.

OUR ASSOCIATION

NEW SECTION FOR THE JOURNAL

The report of a special committee on publication policy was published on page 449 of the March 1960 JOURNAL. This committee, of which D. V. Josephson is Chairman, recommended the publication of a section entitled Interpretive Summaries of Papers. The Journal Management Committee, of which J. T. Reid is Chairman, and the Board of Directors, headed by President A. C. Fay, approved the recommendation by a majority vote, to be made effective January 1, 1960. The objectives of this new section are to broaden our publication approach, to make our Journal more acceptable to the present membership and to industry, and more attractive to potential members.

The Interpretive Summaries are intended for reproduction in farm and in trade journals. They should be written in terms that will be understood by personnel with operating and supervising responsibilities in the dairy industry. Objectives for research should be defined. Important results should be clearly stated. Research tools and technical terms should be explained, insofar as is possible. These summaries should not exceed 8 to 10% of the length of the original paper.

The summaries in the JOURNAL for January and February will serve as guides to authors. Attention is directed to the one by Campbell and Phelps on page 286 and to the one by Schmidt on page 291 of the February JOURNAL.

The editor will welcome comments and criticisms of this new section. It will be of interest to learn if this section achieves the stated objectives, and if this is a logical function for a scientific journal.

—E. O. HERREID
Editor-In-Chief

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